



Science

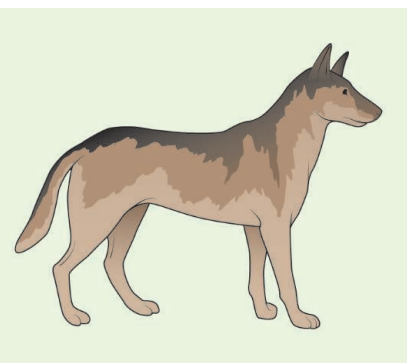
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EXPLORING
MARTIAN HABITABILITY

 AAAS



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COVER

Eroded landscape of Yellowknife Bay, Gale crater on Mars. Sheepbed mudstone is seen in the foreground, ~4 meters distant from the Curiosity rover camera that took the photo; Gillespie sandstone is in the middle field. The foothills of Mt. Sharp (upper left), ~20 kilometers distant, are Curiosity's ultimate destination. Exploration of this region by the Curiosity rover offers evidence of an ancient, potentially habitable environment. See the special section beginning on page 386 and at www.sciencemag.org/extra/curiosity.

Image: NASA/JPL-Caltech/Malin Space Science Systems

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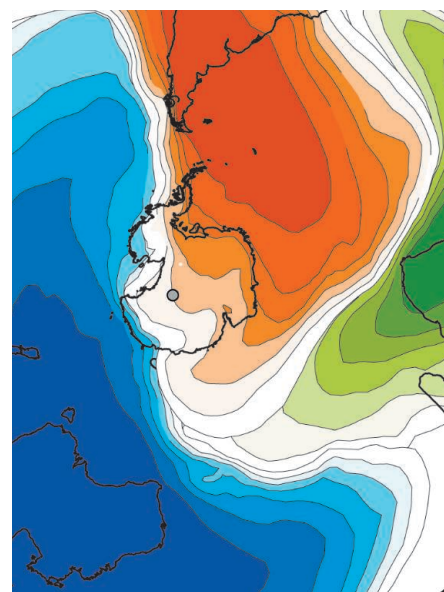
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SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Copyright © 2013 by the American Association for the Advancement of Science. The title SCIENCE is a registered trademark of the AAAS. Domestic individual membership and subscription (51 issues): \$149 (\$74 allocated to subscription). Domestic institutional subscription (51 issues): \$990; Foreign postage extra: Mexico, Caribbean (surface mail) \$55; other countries (air assist delivery) \$85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. Printed in the U.S.A.

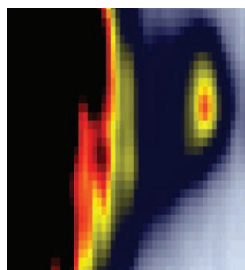
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A View from the Middle

The intuitive way to study a bimolecular reaction is to induce a collision between separate reagents and then track the ensuing events. Crossed molecular beam studies have revealed the quantum mechanical details of numerous systems in this fashion. **Otto *et al.*** (p. 396, published online 9 January) applied a more recent approach of starting in the middle of the $F + H_2O \rightarrow HF + OH$ reaction trajectory, postcollision, by photodetaching an electron from a stabilized complex of water and a fluoride ion, and then tracking the fate of the neutral fragments.

Copper-Oxide Superconductors

Copper-oxide superconductors have a complex electronic structure. A charge density order has been observed in two cuprate families; however, it has been unclear whether such an order exists in Bi-based compounds (see the Perspective by **Morr**). **Comin *et al.*** (p. 390, published online 19 December) and **da Silva Neto *et al.*** (p. 393, published online 19 December) address this question in single-layer and double-layer Bi-based cuprates, respectively. For both families of materials, surface measurements by scanning tunneling



spectroscopy agree with bulk measurements obtained through resonant elastic x-ray scattering, which suggests the formation of short-range correlations that modulate the charge density of the carriers over a range of dopings. Thus, charge ordering may represent a common characteristic of the major cuprate families.

Noise in Motion

A large earthquake along the southern San Andreas Fault has the potential to cause serious damage to the city of Los Angeles, USA. Earthquake simulations in this region, which lies in a sedimentary basin capable of amplifying shaking, predict strong ground motion but they lack validation with observational data. **Denolle *et al.*** (p. 399) developed an independent method to predict ground motion using virtual earthquakes and information gleaned from background seismic noise. This ambient seismic field—generated by sources such as the

Living Technicolor>>

Color vision is generally carried out through the number of photoreceptor types found in the retina. The mantis shrimps (stomatopods) can have up to 12 photoreceptors, far more than needed for even extreme color acuity. **Thoen *et al.*** (p. 411; see the Perspective by **Land and Osorio**) conducted paired color discrimination tests with stomatopods and found that their ability to discriminate among colors was surprisingly low. Instead, stomatopods appear to use a color identification approach that results from a temporal scan of an object across the 12 photoreceptor sensitivities. This entirely unique form of vision would allow for extremely rapid color recognition without the need to discriminate between wavelengths within a spectrum.



oceans and atmosphere—produces differences in ground motion in the Los Angeles Basin compared to simulations, but suggests that locally shaking could on average be 3 times larger than the surrounding areas.

Observing the Messenger

In order to elucidate the dynamics of individual components in the cell, single-molecule technologies are being developed (see the Perspective by **Akbalik and Schuman**). **Park *et al.*** (p. 422) used a mouse expressing fluorescent β -actin messenger RNAs (mRNAs) to visualize mRNA movements in living cells and tissues. **Buxbaum *et al.*** (p. 419) showed that neurons contain β -actin mRNAs and ribosomes packaged in a dense structure, impenetrable by oligonucleotide probes. This effectively masks the mRNAs until neuronal stimulation exposes the mRNA and ribosomes briefly, presumably reflecting the local stimulation and translation involved, for example, in the generation of memories.

Sensing HIV

The depletion of quiescent CD4⁺ T cells from lymphoid organs is a major event contributing to the development of AIDS. The accumulation of incomplete HIV DNA transcripts in the cytoplasm of these cells, which do not themselves become productively infected, is somehow sensed, which triggers cell death. **Monroe *et al.*** (p. 428, published online 19 December; see the Perspective by **Gaiha and Brass**) now identify the host DNA sensor as

interferon- γ -inducible protein 16, which senses viral DNA and activates pyroptosis, an inflammatory cell death pathway.

Dust in the Sea

The effect of windblown dust on marine productivity in the Southern Ocean is thought to be a key determinant of atmospheric CO₂ concentrations. **Lamy *et al.*** (p. 403) present a record of dust supply to the Pacific sector of the Southern Ocean for the past one million years, derived from a suite of deep-sea sediment cores. Dust deposition during glacial periods was 3 times greater than during interglacials, and its major source region was probably Australia or New Zealand.

Breaking Tumor Dogma

Canine transmissible venereal tumor (CTVT) is an unusual form of cancer because the infectious agent is not a virus or bacterium but the tumor cells themselves, which are passed from one dog to another during coitus. To explore the molecular features of the tumor and its possible origins, **Murchison *et al.*** (p. 437; see the Perspective by **Parker and Ostrander**) sequenced the genomes of two CTVTs and their host dogs, one from Australia and one from Brazil. Although CTVT has acquired a massive number of genomic alterations, including hundreds of times more somatic mutations than are normally found in human cancers, the tumor cell genome has remained diploid and stable. Indeed, CTVT may first have arisen in a dog that lived more than 10,000 years ago.



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Challenges for New ERC President

THIS MONTH, JEAN-PIERRE BOURGUIGNON BEGAN HIS TENURE AS PRESIDENT OF THE EUROPEAN Research Council (ERC). This is a daunting task for many reasons: The growing ERC budget requires that it be carefully positioned within a complex array of European funding opportunities; embedding the presidential role into the ERC executive agency in the European Commission's Directorate-General for Research and Innovation potentially reduces ERC's independence; and variable success rates for acquiring ERC funding are concerning, and research expectations will need to be managed.

The ERC provides competitive funding for researchers worldwide, with no thematic top-down steers. Most awards are allocated through starting grants for early-career scientists and advanced grants for senior scientists. "Synergy" and "proof-of-concept" schemes have been added recently, although Bourguignon acknowledges that they have had mixed success.* The 60% increase in the ERC budget to around €13 billion (€1.8 billion per year from 2014 to 2021) has been welcomed by researchers, particularly because the ERC is regarded as relatively unbureaucratic, focused on excellence, and encouraging of innovative ideas.

Simultaneously, there are many excellence-focused national funding programs, and the ERC needs to meld sensibly into this complex environment. ERC support for basic research should complement and not replace national project-based competitive funding. In many respects, the ERC has been modeled on best national practices, and one priority is that the ERC discuss future strategic directions with the national funding agencies that are represented through Science Europe. Funding for basic research is considerably larger from national agencies. The Deutsche Forschungsgemeinschaft alone, only one of the major agencies in Germany, has a 2014 budget of €2.78 billion for bottom-up research; any thematic decisions result from consultation with the German research community. With such opportunities for "curiosity-driven" research in each European country, it is evident that the ERC needs to continue to articulate its added value. For example, unlike national schemes, it is open to researchers worldwide, but the number of such grantees remains lower than might be hoped.

Another challenge is that Bourguignon's predecessor, Helga Nowotny, provided high-profile leadership that benefited from her relative independence from the European Commission. Although the decision to embed the new president within the ERC Executive Agency in the Commission—merging the Secretary General and presidential functions—may provide administrative benefits, Bourguignon's ability to represent the voice of the research community in such a political role will be questioned, which he recognizes.*

There are also contentious practical challenges, acknowledged by the ERC. Low overall success rates (8.6% for starting grants and 11.8% for advanced grants in 2013) and the gender bias in applications and success run the risk of disillusioning applicants, especially were these to fall further. Moreover, although the ERC budget has increased, the 2014 budget is fairly similar to that in 2013, due to the ramped funding profile during the Seventh Framework Programme. This means that opportunities are not substantially greater this year and that "expectations management" will be necessary, such as the recent tightening of rules for resubmitting applications. Managing interdisciplinary applications will continue to be challenging, because ERC funding is allocated through three discipline-specific budget lines, and the review process requires experts in evaluating such multifaceted proposals. The low success rates for starting and advanced grants in the social sciences and humanities raise questions about whether the funding caps in these areas need revisiting. And with more than half of the 2012 starting grant awards secured by the United Kingdom, Germany, France, and the Netherlands alone, there is concern about researcher concentration, especially because ERC grants are portable between institutions.

ERC is but one part of an excellence-focused European funding environment. By focusing the ERC on its strengths, I am confident that Jean-Pierre Bourguignon will manage this exciting new funding phase with aplomb.*

— Paul Boyle

10.1126/science.1250820

*<http://news.sciencemag.org/europe/2013/12/europes-new-basic-research-chief-ready-defend-fundamental-science>.



CLIMATE SCIENCE

More Than We Thought

One of the most worrying impacts of climate warming is the sea-level rise caused by melting or collapse of the polar ice sheets. The Antarctic Ice Sheet contains enough water to raise sea level by roughly 60 m were it to melt completely. Most of the work done to determine the influence of warming on the Antarctic Ice Sheet has focused on the West Antarctic Ice Sheet, which is thought to be the most unstable portion with respect to warming. Fogwill *et al.* consider the East Antarctic Ice Sheet (EAIS), which contains 90% of Antarctic ice, using a computer model to examine how much of that region may have melted or collapsed 135,000 to 116,000 years ago during the last interglacial, when the global average air tempe-

rate was about 2°C higher than it is now (a potential analog for the warmer climate of the next century). They focus particular attention on the effects of the Southern Hemisphere westerly winds on Southern Ocean circulation and the dynamics of the Antarctic ice sheet, concluding that the EAIS may have made a significantly greater contribution to sea-level rise over that period than currently is believed, with the implication that projected changes in the climate of the southern hemisphere may constitute a more serious threat to the future stability of the EAIS than has generally been appreciated until now. — HJS

J. Quat. Sci. 29, 91 (2014).

ECOLOGY

Count Consumers

Invertebrate herbivores are an important component of tropical forest ecosystems, and new data from forests in Peru quantify their role in biogeochemical cycling. Metcalfe *et al.* surveyed patterns of herbivory in forests along an elevational transect from 300 to 3000 m in the Andes, over a full seasonal cycle, to calculate rates of herbivory and its effect on pathways of nutrient cycling (particularly N, P, and C). Herbivore activity was greater at lower elevations and higher temperatures and at high concen-

tropical forests, as changes in environmental conditions lead to shifts in abundances of herbivore populations. Complex as they may be, the activities and effects of consumers should be incorporated into global vegetation models in order to accurately predict the likely consequences of global change. — AMS

Ecol. Lett. 10.1111/ele.12233 (2013).

BIOMEDICINE

Happy Gut, Happy Mouse?

Growing evidence suggests a role for perturbations in the gut microbe balance (dysbiosis)

and gastrointestinal (GI) complications in autism spectrum disorders (ASD). Altered microbial composition is observed in autistic individuals, and subsets of these patients exhibit improved symptoms with antibiotic treatment or when placed on restricted diets. A strong correlation has also been observed between GI status and autism severity. Recent animal studies have revealed that the gut microbiota modulate a variety of behaviors, including anxiety-like and emotional behaviors. Indeed, the microbiota affect neurotrans-

mitter levels, brain gene expression, and vagal nerve activation. Dysbiosis may contribute to allergy, autoimmune disease, obesity, and inflammatory bowel disease. Hsiao *et al.* were able to ameliorate behavioral abnormalities in a mouse model of ASD by treating animals with

the human gut bacterium *Bacteriodes fragilis*. The treatment restored intestinal barrier integrity, modulated serum metabolites, and helped with behavioral symptoms. — SMH

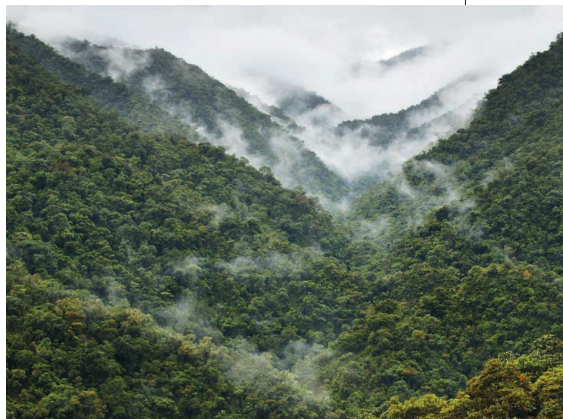
Cell 155, 1451 (2013).

BIOMATERIALS

Glue for the Heart

The surgical attachment or closure of biological tissues typically requires sutures, staples, or glue. Both sutures and staples can cause additional tissue damage, particularly in damaged or juvenile tissue. Existing adhesives can be washed away under in vivo conditions or may not be strong enough to withstand large forces, such as those found in the cardiovascular system. Lang *et al.* designed a hydrophobic prepolymer based on poly(glycerol sebacate acrylate) (PGSA), which is composed of biocompatible and biodegradable components, and mixed in a photoinitiator. This adhesive was readily spread over a surface and provided a strong bond, even with wet tissue that had been in contact with blood, upon cross-linking during a few seconds of exposure to ultraviolet light. Tests in rats showed that the adhesive could be used to attach a polymer patch to seal up a left-ventricle wall defect, with similar success rates as a suture control group. A second set of tests looked at the closure of ventricular septal defects within a pig heart. Of the four animals that were tested, two were monitored for 24 hours, while the other two were exposed to accelerated heart rate and blood pressure, and in all cases the adhesive held firm. — MSL

Sci. Transl. Med. 6, 218ra6 (2014).



trations of foliar P, with up to 20% of foliar productivity diverted to herbivores over the year. Herbivory also contributed an unexpectedly high proportion of N and P inputs to the soil. These patterns in turn may have implications for future C cycling and sequestration in

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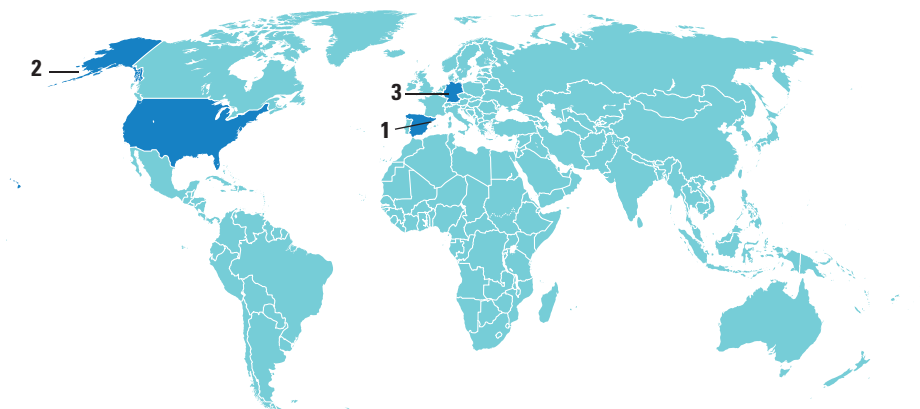


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AROUND THE WORLD



Barcelona, Spain 1

Director Leaves Research Center In State of Uncertainty

A pioneering Spanish stem cell center is surrounded by questions in the wake of its leader's departure. Developmental biologist Juan Carlos Izpisua Belmonte last week stepped down as the director of the Center of Regenerative Medicine in Barcelona (CMRB), which he helped create almost a decade ago with support from the Catalan and Spanish governments. Izpisua Belmonte was personally involved in most of the research projects at the center while also a professor at the Gene Expression Laboratory at the Salk Institute for Biological Studies in San Diego, California.

Press reports, including one in the Spanish newspaper *El País* that broke the story, suggest that he may try to take many ongoing projects with him and that this could reduce CMRB to an empty shell. Andreu Mas-Colell, minister of economy and knowledge of the government of Catalonia, confirms that there are concerns over patents and that government lawyers will look into the issues. Izpisua Belmonte did not respond to requests for comment, but many expect him to take up research at the Salk Institute full-time. http://scim.ag/_CMRB

Science LIVE

Join us on Thursday, 30 January, at 3 p.m. EST for a live chat with experts on **giving patients and research participants access to their raw personal data**. <http://scim.ag/science-live>

Bristol Bay, Alaska 2

Planned Mine Would Put Salmon at Risk

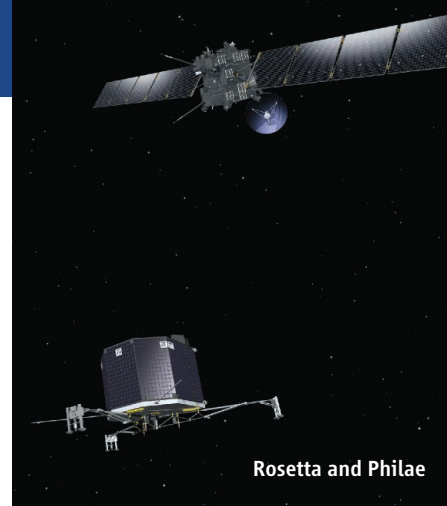
A massive proposed copper and gold mine in Alaska would damage the world's biggest sockeye salmon fishery, worth \$480 million a year, according to a 15 January assessment released by the U.S. Environmental Protection Agency (EPA). It estimates that the planned Pebble Mine would destroy up to 150 kilometers of salmon streams and 2165 hectares of wetlands and lakes in the Bristol Bay watershed. Further impacts could include



Wetlands near Bristol Bay

changes to streamflow, leaking mine waste, and toxic pipeline failures. Pebble Limited Partnership (PLP) CEO John Shively said in a statement that EPA's evaluation was rushed and "very flawed," in part because it didn't consider advanced engineering and mining practices.

A bigger setback for the mine is that Anglo American, a half-owner, withdrew from the project in September, implying the financial risk was too high. In December, another major partner, Rio Tinto, announced that it is also considering pulling out. PLP has spent \$600 million on studies to plan for the mine, but has not yet applied for key permits.



Rosetta and Philae

Darmstadt, Germany 3

European Comet-Chaser Emerges From Hibernation

The European spacecraft Rosetta has roused from a 957-day nap and is preparing to close in on its target: the comet 67P/Churyumov-Gerasimenko. Rosetta had been put into hibernation as sunlight became too dim to sustain its solar-powered systems, but on 20 January it successfully signaled to the European Space Agency (ESA) control center in Germany. "This was one alarm clock not to hit snooze on," said Fred Jansen, ESA's Rosetta mission manager, in a statement.

The unprecedented mission will ride along with the comet for more than 18 months during its close approach to the sun. Samples from the comet's surface and atmosphere could offer insight into the formation of planets and suggest whether comets could have supplied Earth with water. Scientific observations will begin in May, and Rosetta will dispatch its lander, Philae, onto the comet's surface in November.

http://scim.ag/_awake

NEWSMAKERS

'Nobel of the Geosciences' Goes to Mountain Man

The Royal Swedish Academy of Sciences last week awarded the 2014 Crafoord Prize in Geosciences—and about \$620,000 in prize money—to geophysicist **Peter Molnar**, 70, of the University of Colorado, Boulder. Trained as a seismologist, Molnar has worked with everyone from geologists to atmospheric scientists and says he has used "any technology I can get my hands on" to understand what happens when continents collide and how the resulting high places affect climate downwind.

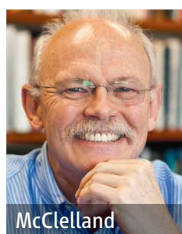


Molnar

The academy unveils one Crafoord prize per year, in astronomy and mathematics, biosciences, polyarthritis, or geosciences. This year's honoree says he never misses a chance to venture into the high, cold places of the Himalayas and Tibetan Plateau, even if he's only carrying rock samples for his teammates. "I enjoy so much of what I do," he says. "We've come a long, long way the last 40 years, but then damn wrenches get thrown" into the developing picture of how colliding continents behave. He sees no end to the fun.

Psychologists Nab First National Academy Prize

The U.S. National Academy of Sciences (NAS) inaugural Prize in Psychological and Cognitive Sciences goes to pioneers in neural network modeling and the developing brain. Stanford University psychologist **James McClelland** is known for his role in describing "parallel distributed processing," a model for how cognition arises from an interconnected network of neurons. His co-recipient, cognitive psychologist **Elizabeth Spelke** of Harvard University, has focused on infants and children to reveal how the young brain represents concepts such as numbers and spatial relationships. McClelland and Spelke will each receive \$200,000 for their "significant contributions to our understanding of how the brain works," said NAS President Ralph J. Cicerone in an announcement of the new biannual prize last week.



McClelland



Spelke

NOTED

>Chinese researcher **Chen Yingxu** was sentenced to 10 years in prison on 7 January after an audit uncovered that he had embezzled about \$1.55 million from a massive water-quality research grant (*Science*, 9 August 2013, p. 598). Chen's lawyer has blasted the sentence as too harsh, claiming that his client had returned the money before the audit occurred.



Three Q's

The improbable pioneer of basketball diplomacy has an equally eccentric scientist in his entourage. **Joseph Terwilliger**, 48, a statistical geneticist at Columbia University (and sometime tuba player, Lincoln impersonator, and competitive hot dog eater), won a meeting with Dennis Rodman as a prize in a charity auction last April. Because Terwilliger speaks Korean and has connections in Pyongyang, Rodman's camp enlisted him to help coordinate the Hall of Famer's next visit. Terwilliger shared his recent experiences with *Science*.

Q: Last July, you spent 3 weeks teaching at Pyongyang University of Science and Technology. What was that like?

◀ **Kindred spirits.** Terwilliger and Rodman.

J.T.: I viewed my role there as one of showing the positive side of the American people to a population who has heard mostly negative stereotypes about us. [Students said they] needed to think twice about their opinions of American people as a result of our interactions. They said the very same thing about their experiences viewing Dennis Rodman's visit to their country in February of 2013.

Q: Has traveling with Rodman changed your life?

J.T.: No, I have kept a very low profile. Sure, my photo is all over the place, but most people just ask "Who is the crazy looking bearded guy with Dennis?" I was just there to assist him, to help with translation.

Q: Do you see an opportunity to engage the North Koreans through science diplomacy?

J.T.: I hope to have more opportunities to interact with the North Koreans on a scientific level—in fact that was a large part of my motivation to get involved with Rodman's efforts as a way to build contacts, connections, and trust, so something positive could happen down the road.

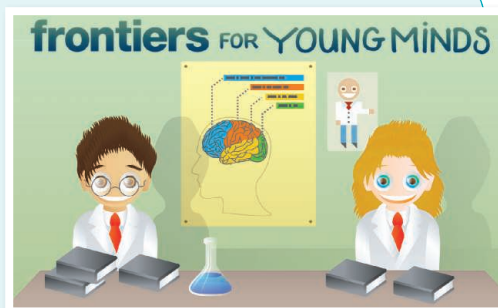
Extended interview at <http://scim.ag/Rodtrip>.

Random Sample

Young Editors Ask Neuroscientists: 'Why Should I Care?'

While sitting on a scientific review panel, University of California, Berkeley, neuroscientist Robert Knight once "got so profoundly bored" that he brainstormed ways to enliven the process. What if kids were in charge of reviewing journal articles, he wondered? Although it took a few years, Knight has now convinced the Nature Publishing Group (NPG) to launch an online research journal called *Frontiers for Young Minds* which is not only geared toward kids, but edited by them. A team of nearly 50 grown-up neuroscientists volunteer to write about topics ranging from how we see color to why we sleep, and editors ages 8 to 18—assisted by mentors, often graduate students—wield the red pens.

More than a dozen articles have already been published and, Knight says, "not one scientist has complained about something the kids wanted to fix." That's in spite of some pretty tough criticism. "I can see that it shows something about how the brain works," wrote one uncompromising young editor, "but it is not obvious how this knowledge will help with anything." So far, most editors are the children of scientists, but Knight says that the journal will launch efforts to attract a broader range of students, including kids from disadvantaged backgrounds, at the USA Science & Engineering Festival in April. He plans to create Spanish and Chinese versions of the journal, and NPG may also launch parallel projects for different scientific disciplines. "My wife can tell when I'm working on the journal because I'm happy," Knight says.





CLINICAL RESEARCH

Divulging DNA Secrets Of Dead Stirs Debate

When Gloria Petersen began hunting for gene mutations behind pancreatic cancer, she knew that nearly all of the more than 3300 patients in her study would die—and one by one, most have. But the posthumous knowledge they left behind put Petersen, a genetic epidemiologist, in an uneasy position. Several of the mutations she found, such as those in the gene *BRCA2*, can set in motion breast cancer, ovarian cancer, or melanoma—tumors that, unlike those in the pancreas, can be prevented or caught early. It dawned on Petersen that the DNA in her freezers brimmed with information relevant to the living—her patients' families.

Was she obliged to alert adult children, siblings, or cousins to consider genetic testing? Was it even legal—not to mention ethical—to share health information obtained through a research study without a patient's consent? "That," she says, "was my dilemma."

Petersen, who works at the Mayo Clinic in Rochester, Minnesota, is among the first trying to glean answers to these questions. With help from a legal scholar and a medical anthropologist, and a \$2.4 million grant from the National Cancer Institute in Bethesda, Maryland, she's now evaluating how to return genetic information to relatives of a research participant who has died.

Others are facing similar predicaments. At a major U.S. cancer center, for example, researchers recently unearthed a cancer gene mutation in banked tissue from a deceased study participant. After a round of soul-searching and legal discussions about

whether and how to share the news with family, they are leaning toward reaching out.

The problem is sure to grow. "Think of all the biorepositories sitting around with information, and their source individuals are dead or dying," says Susan Wolf, a law professor and bioethicist at the University of Minnesota, Twin Cities, who is collaborating with Petersen. "We're talking about medical information of potential significance to

"We're talking about medical information of potential significance to survivors."

—SUSAN WOLF, UNIVERSITY OF MINNESOTA

survivors." There might be clues to gene-drug interactions, and genetic risks factors for everything from Alzheimer's disease to cancer.

In the United States, once a person dies their "personal representative"—often, by state law, the executor—has access to their health information. If that information is relevant to family members, doctors may advise the representative to disseminate the news. But the law is less clear when it comes to research results, and scientists don't typically feel the same obligations to their study volunteers as a physician might to a patient. It's also unclear whether research findings become part of a person's official medical record, Wolf says.

More and more, researchers are thinking ahead, having study participants specify who,

if anyone, can access their genetic results. Memorial Sloan-Kettering Cancer Center and a coalition of Dutch cancer centers, for example, recently began, or plan to begin, asking participants who should receive results if they are "no longer available."

Leslie Biesecker, chief of the Genetic Disease Research Branch at the National Human Genome Research Institute in Bethesda, Maryland, considered something similar for his study, ClinSeq. It's returning a broad range of genetic findings to more than 1200 people.

But he eventually decided not to ask for guidance from participants, because family dynamics can change. Instead, he relies on the executor, normally a trusted family member, to convey what the deceased would have wanted. "We're very hesitant to paint ourselves into a corner by a designation made years ago that may no longer be valid," he says.

"You're in the real and messy world of families," Wolf agrees.

So should a research subject's wishes be honored after death at all costs, even if that means their relatives won't learn about a disease that's potentially preventable? "Painfully, yes," says Emile Voest, medical director of the Netherlands Cancer Institute. But Wolf hopes to strike a balance between the wishes of the now-deceased volunteer and the medical needs of their survivors. In some respects, she says, the answer may be culturally specific and depend on how different countries value family versus individual rights.

As part of her collaboration with Wolf and Barbara Koenig, a medical anthropologist at the University of California, San Francisco, Petersen recently surveyed 3000 people with pancreatic cancer and their families and 3000 controls, seeking input about privacy and information-sharing after death in different scenarios. Relatives nearly always say, "If you know that I have a *BRCA2* mutation in my family, you have an obligation to let me know," Koenig says. But depending on how and with whom information is shared, disclosure may be "deeply in conflict" with the Health Insurance Portability and Accountability Act passed in 1996, as well as state privacy laws.

Perhaps the knottiest question when a study volunteer is dead is how to return information to relatives if the researcher doesn't know whether they want it. Petersen, Koenig, and

Wolf are now debating how best to go about this for *BRCA2*, one of four key genes in which Petersen found mutations linked to pancreatic cancer. The mutations in these genes turned up in 73 of the deceased volunteers, meaning that hundreds of family members may also carry them. “Do you call people up and say, ‘We know something, do you want to know?’ ” Koenig wonders. Another option is to mail a newsletter to all families, offering general

information about the genes in question—but this, the researchers worry, may fail to convey sufficient urgency.

Other institutions, convinced they can’t override informed consent agreements signed with research volunteers, are trying to keep themselves out of sticky situations. At Sloan-Kettering, researchers studying samples from donors who may have died must anonymize the samples if they’re testing for inherited

gene mutations. Dutch cancer centers normally do the same, Voest says.

Wolf and her colleagues plan this fall to share draft guidance for the community about when and how to return genetic results to next of kin. “I’ve been sitting on it for a long time with these families,” Petersen says, referring to the DNA stored in her lab. Now, she’s hoping to get the word out.

—JENNIFER COUZIN-FRANKEL

U.S. SPENDING

NIH Is Losing Its Funding Edge, 2014 Budget Suggests

The National Institutes of Health (NIH) receives nearly one-half of what the federal government spends on basic research. But when it’s time to dole out more dollars, NIH may no longer be the first among equals.

A December agreement to ease the pain of last year’s across-the-board spending cuts known as sequestration freed up some \$22 billion in additional discretionary funding for domestic programs. But in a break from the past, agencies supporting the physical sciences and engineering research did much better than NIH in the 2014 budget that was enacted last week (*Science*, 17 January, p. 237). And analysts say the pattern could repeat itself in the 2015 fiscal year, when legislators have no more to spend.

Although science advocates typically campaign for increases across all fields, NIH has dominated spending ever since Congress began an unprecedented 5-year budget doubling in 1998. In fact, NIH’s 2004 budget was larger than the entire federal investment in research in 1988.

The doubling, pushed by a handful of powerful legislators who capitalized on a booming economy and on the traditional bipartisan support for science within Congress, actually caught advocates by surprise. In 1995, then-NIH Director Harold Varmus warned researchers to prepare for a “steady-state environment that is likely to persist for the foreseeable future.” But once achieved, the doubling raised expectations among biomedical researchers that NIH would receive the largest share of any extra

money spent on research. That attitude was reinforced by the \$10 billion bonanza NIH reaped in the 2009 stimulus bill.

Federal policymakers have tried to correct that imbalance. In 2006, then-President George W. Bush proposed a similar doubling for the National Science Foundation (NSF) and two other key agencies supporting the physical sciences. The next year, Congress overwhelmingly passed the America COMPETES Act, which laid out that path. But the money never showed up.

Meanwhile, NIH’s budget has languished since doubling ended. Sequestration took a \$1.5 billion bite out of its 2013 budget, and this month Congress restored \$1 billion, giving it a 3.5% bounce. In contrast, lawmakers gave double-digit increases to some agencies that support the physical sciences.

physical sciences and engineering retains rare bipartisan appeal,” Sarewitz says. “Everyone still loves NSF, and connects physical sciences to jobs, which is what everyone worries about now, as they should, more than health.”

Other factors are also in play. NIH faces stiff competition within its appropriations subcommittee from other social programs, and some veteran members may be suffering from postdoubling “NIH fatigue.” This year’s budget also addresses several pressing needs at NASA and across the Department of Energy’s national labs that lawmakers apparently felt couldn’t wait.

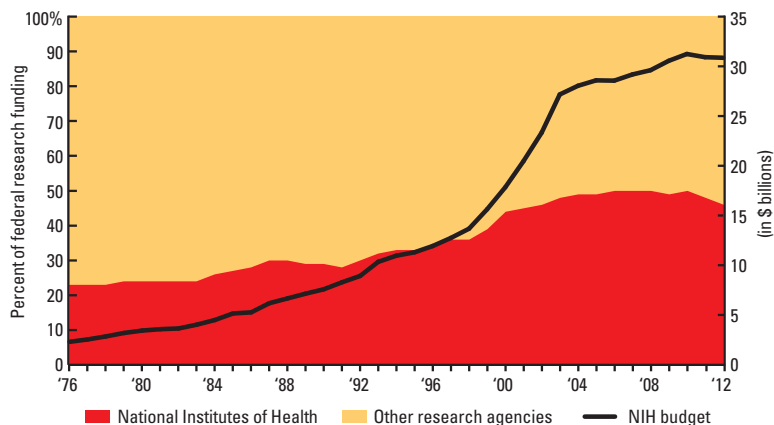
President Barack Obama’s State of the Union address next week marks the start of the 2015 budget process. But biomedical research advocates aren’t backing down.

“I could make the same argument for the life sciences about job creation,” says former Representative John Porter (R-IL), a leader of the doubling push. Now chair of the lobbying group Research!America, Porter says the path toward more research funding is for Congress to revise the 2011 budget agreement that triggered the across-the-board cuts. “The best thing for science is to get rid of the sequester and reform entitlement programs,” he says, echoing an argument made by Obama and congressional Democrats.

Nobody expects Congress to get serious about the 2015 budget until after voters elect a new Congress in November. Then NIH’s budget prowess will be put to a stern test.

—JEFFREY MERVIS

Birth of a Biomedical Science Giant



NIH’s rising share of federal research spending was fueled by a 5-year doubling of its budget.

Dan Sarewitz of Arizona State University, Tempe, thinks legislators are more worried about the economy and unemployment than they were 15 years ago and don’t necessarily see NIH as a job creator. “Basic research in the

MATERIALS SCIENCE

Nano-Imaging Feud Sets Online Sites Sizzling

Scientific controversies often sort themselves out as new data roll in. But a decade-old dispute in nanoscience shows no sign of letting up. Researchers on both sides are claiming that recently published papers settle the debate in their favor, while one is charging his opponents with resorting to an electronic bullying campaign.

The clash dates back to a 2004 *Nature Materials* paper in which researchers led by Francesco Stellacci, then at the Massachusetts Institute of Technology (MIT) and now at the Swiss Federal Institute

spot signatures consistent with Stellacci's stripes seals the deal, says Biscarini, a chemist and expert in scanning microscopy. "In my mind the controversy is over."

Stellacci's critics—chiefly U.K.-based STM experts Raphaël Lévy of the University of Liverpool and Philip Moriarty of the University of Nottingham—were quick to respond. All along they have contended that Stellacci and his colleagues made basic mistakes in their imaging studies. For example, they say, the original "stripes" were created by electronic feedback in the STM.

what kids that commit suicide go through." Instead of engaging in such "unethical and unprofessional" conduct, he says, the skeptics should go through the normal channels of peer review and publish their data in journals so the scientific process can work through the issues.

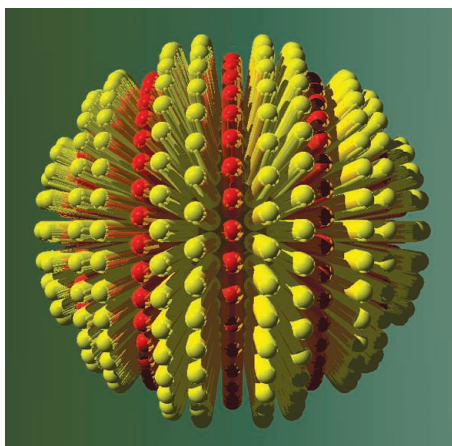
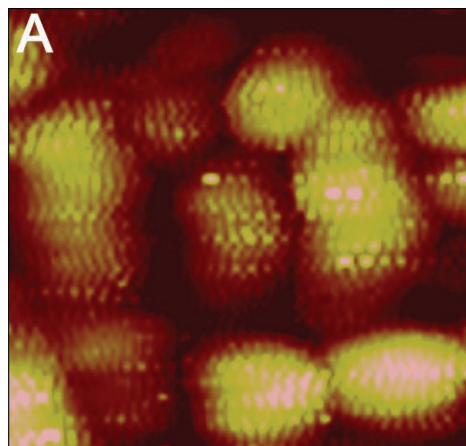
But the critics say their adversarial approach is normal science at work and that researchers should not hide behind the cloak of peer review. "I have no time at all for this argument," Moriarty says. "If you're publicly funded, tough. Get out there and face your critics." He and Lévy say they were forced to go online in this case because the peer-review process was far too slow. Lévy says he had to wait 3 years to get a manuscript published in response to the original *Nature Materials* paper. "It shows there are serious problems with the way science is evaluated [using peer review]," Lévy says. He adds that he has "no personal conflict" with Stellacci and would post unedited any rebuttal or commentary Stellacci cared to send. Stellacci, however, says he refuses to be drawn into an unending scuffle with opponents who misrepresent his work.

Kelly agrees with critics that the stripes in the original paper "look like an imaging artifact," but he and others say the jury is still out on more recent reports. The bottom line, Kelly says, is that trying to take images of stripes just two to three molecules wide on a tiny curved surface pushes the current limits of nanoscience. "They are trying to do a really hard measurement," he says.

As scientific imbroglis go, this one pales beside such once-raging controversies as cold fusion, arsenic-based life, and credit for the discovery of HIV. "This can be seen as a minor storm in a nano teapot," Moriarty acknowledges. But, he says, he feels compelled to respond to prevent mistakes from proliferating through the literature.

Now, Moriarty and Lévy are getting a taste of their own maelstrom: Their recent paper touched off a heated debate on PubPeer, in which a commenter labeled "Unregistered Submission" has repeatedly picked apart their science. Moriarty suspects either Stellacci or one of his students or co-authors. (Stellacci denies any involvement.) "I would prefer to get rid of anonymous comments, and I am glad that Francesco Stellacci and I have that in common," Moriarty says. For now, as the tempest roils on, that's about all they agree on.

—ROBERT F. SERVICE



See anything? An image of gold nanoparticles from a 2004 paper (left) showed features that Francesco Stellacci interprets as organic "stripes" (model, right) but that critics attribute to STM feedback.

of Technology in Lausanne (EPFL), reported that they had created gold nanoparticles with stripes of two different organic compounds, which the team imaged using a scanning tunneling microscope (STM). Stellacci says such stripes could help nanoparticles enter cells and thus might be useful for delivering medicines or imaging agents. But critics took to blogs, arguing that the stripes were likely artifacts of Stellacci's attempt to image features at the very limit of resolution.

Researchers on the outskirts of the fray are bewildered at the intensity of the dispute, saying the scientific stakes are modest. "I'm stunned at how long this has been going on," says Kevin Kelly, a scanning tunneling microscopist at Rice University in Houston, Texas.

Four pro-stripe papers by Stellacci and other researchers have stoked the debate. Fabio Biscarini of the University of Modena and Reggio Emilia in Italy is the lead author of one, co-authored with Stellacci and published in *Langmuir* late last year. The fact that four labs using a variety of techniques

Now, in an article posted on the arXiv online physics preprint server and submitted to *PLOS ONE*, they charge that the new papers are riddled with cherry-picked images, patterns imposed on what is essentially noise, and other mistakes that undermine the authors' interpretation of the data.

Just as fierce is the metadisperse over the way the critics of Stellacci's experiment have waged their campaign. Stellacci notes that his critics have made four formal misconduct charges against him: two to MIT and one each to EPFL and the journal *ACS Nano*. Investigators cleared him in all four cases. What's more, apart from a couple of papers in journals, the critics have posted most of their denunciations online in blogs, on Twitter, and in anonymous comments on the postpublication criticism website PubPeer. Numerous harsh critiques of Stellacci's work, both anonymous and attributed, have appeared on a blog Lévy runs on his research group's website.

"I have been subject to chemical cyber-bullying," Stellacci says. "I understand

INTELLECTUAL PROPERTY

Historic Patent on Embryonic Stem Cells Faces Scrutiny

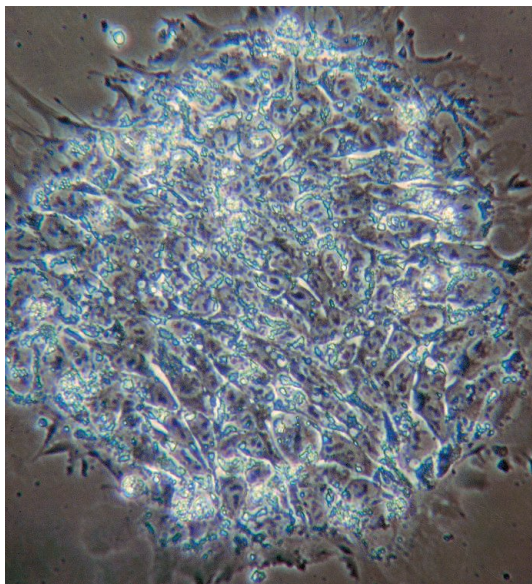
Human genes cannot be patented because they are a product of nature, the U.S. Supreme Court concluded in landmark ruling this past June. So why allow anyone to patent human stem cells, also arguably a product of nature?

That question is now before a U.S. court in a suit brought by Consumer Watchdog (CW), an advocacy group in Santa Monica, California. The new lawsuit aims to invalidate a 2006 patent awarded to biologist James Thomson, who in 1998 was the first to isolate and culture a long-sought population of stem cells from human embryos.

If the challenge succeeds, and a critical initial hearing on it is expected this month in a federal appeals court just below the Supreme Court, it could have broad implications. Over the long run, a win by CW could expand the sweep of the Supreme Court's gene patent ruling and make other biopatents more vulnerable to invalidation, potentially scaring off investors. More immediately, it could cut off a stream of revenue for the Wisconsin Alumni Research Foundation (WARF), an offshoot of the University of Wisconsin, Madison, where Thomson has worked for many years and now heads a regenerative medicine program.

The fight's potential effect on stem cell science, however, is less clear. Many scientists are unconcerned, saying they've moved on from the technology at issue in the case. But patent attorney Konstantin Linnik of the Boston law firm Nutter McClennen & Fish isn't so sanguine: Scientists "often like to think [they're] not the ones" who will be affected by such cases, he says.

CW's challenge reflects how the Supreme Court decision is changing the landscape of biopatent litigation. That ruling specifically invalidated patents on the cancer genes owned by Myriad Genetics of Salt Lake City. The court said that human DNA, even if it has been isolated through a tremendous research effort, still remains a natural product, and thus unpatentable. The court didn't say much about how to apply this new policy, leaving open the possibility that other biological materials fall under a shadow of unpatentability.



In the current case, filed last year, CW claims that stem cells fall squarely under this shadow. Specifically, it targets WARF's U.S. patent 7,029,913, which is due to expire in 2015. But to get the case heard, the group must first clear some hurdles, including proving that it has legal grounds or "standing" to sue—the subject of the upcoming hearing.

WARF argues it does not. CW's members were not harmed in any way by the patent, the foundation says, so they have

views the controversy, but they weren't public as *Science* went to press.

If CW does get into court, it will push the argument that WARF is trying to patent nature. In its briefs, the group also lays out allegations—all made before—that the WARF patent covers work that was somewhat obvious, a modest extension of previous research, and enabled by Thomson's unique access to embryo tissue. They further argue that the invention was not novel, as it was anticipated by other scientists. WARF rejects each of these points in its briefs, noting that Thomson's patent is based on his specific achievement of being the first to isolate and maintain a long-lived culture of human embryonic stem cells. No one denies that, WARF says.

Such jousting doesn't seem to worry many stem cell scientists. In part, they say, that's because most research groups now emphasize or work on only reprogrammed "adult" stem cells, not cells derived from embryos. These adult cells, also called induced pluripotent stem (iPS) cells, aren't as controversial as embryo-derived cells and don't have the same ethical and legal problems. And because they're lab-engineered, many people say iPS cells are not vulnerable to a "product of nature" challenge.

The WARF patent fight is "of no concern at all for iPS cells," contends Nicholas Seay, an attorney who drafted the language for Thomson's patent and is now chief technology officer of Cellular Dynamics, a supplier of iPS cells. The Wisconsin firm, which Thomson helped found, early on made a huge investment of time and money to nail down a "clear title" to every iPS cell line it provides, Seay says. He calculates that the

(12) United States Patent Thomson

(10) Patent No.: US 7,029,913 B2
(45) Date of Patent: *Apr. 18, 2006

(54) PRIMATE EMBRYONIC STEM CELLS

(52) U.S. CL. 435/363; 435/366; 435/373

(75) Inventor: James A. Thomson, Madison, WI (US)

(58) Field of Classification Search 435/366,
435/325; 800/8

New challenge. James Thomson's 2006 patent on human embryonic stem cells (*top*) is back in court.

no standing. But CW argues in legal briefs that it is entitled to a hearing because its petition to invalidate the patent was rejected the U.S. Patent and Trademark Office, and U.S. law guarantees an appeal. CW also argues that the new Myriad decision must be taken into account. The federal court hearing the matter ordered the Justice Department and patent office to file briefs explaining how the Obama administration

company has secured licenses from "dozens" of sources covering "over 700" patents and other intellectual property rights.

Such careful patent planning could become even more important if CW wins its case. Patent attorney Linnik says U.S. courts have been "steadily chipping away at the patentability of biotechnology inventions." As result, he warns that even scientists "cheering on" CW's challenge could come to regret it, as they find it "harder and harder to patent their own inventions."

—ELIOT MARSHALL



War zone. Bombings at the University of Aleppo in January 2013 killed dozens.

was not brave enough to ignite it, but I was waiting for that moment,” she says. Syrian intelligence caught wind of her activism and interrogated her that March. It was early in the uprising, and “they didn’t want to make a big fuss,” she says. “They said, ‘We’ll let it go this time, but be careful.’”

That July, however, a fellow Aleppo professor, Jamal Tahhan, was arrested and detained for 5 months after forming a committee to document peaceful protests; Alachkar says he was tortured. She got the message. Less than a year after getting her neuroscience lab in Aleppo up and running, she shuttered it and left Syria. Aleppo’s cancer research unit, which Al-Mayhany had helped found, also closed after his departure. In mid-2012, the International Center for Agricultural Research in the Dry Areas pulled out of Aleppo, its former grounds looted and “now a war zone,” says Ahmad Sadiddin, an agricultural economist from Damascus who with an IIE grant found refuge at the University of Florence in Italy.

Many professors and students flee without passports across the porous borders to Jordan, Lebanon, and Turkey, where they often end up in refugee camps. That poses a fresh challenge for IIE: “How do you deal with professors in camps who can’t travel to another country?” Goodman asks. To keep the intellectual fires burning, IIE is exploring how to provide materials for 3-week courses that could be taught by scholars in the camps while they await placement in Europe or the United States, Goodman says: “Something they can do other than sit in their tent and worry about how bad things are.”

With the Assad regime and rebels locked in a bloody stalemate, Syrian scholars who have started new lives overseas say they are forced to take a long view. After returning to

Cambridge, Al-Mayhany, now an IIE scholar, co-founded Building the Syrian State, a group that he says advocates “an end to the violence and an end to the despotic regime.” He says he has come to terms with the prospect of never returning to his homeland. Alachkar knows that may also be her fate. “I feel like the situation in Syria is killing me every day,” she says.

—RICHARD STONE

SCHOLAR RESCUE

A Lifeline for Syria’s Science Exiles

For Talal Al-Mayhany, the defining moment of the ill-fated Syrian Spring came in June 2011. As the uprising against the regime was gaining momentum, the young neuroscientist took part in an opposition summit in Damascus. During a press conference, two strangers shouted at Al-Mayhany and others on the podium, calling them traitors and dogs. In the days that followed, attendees were arrested and he received chilling phone threats. A month later, Al-Mayhany fled, returning to the University of Cambridge in the United Kingdom, where he had earned his doctorate.

Many of Al-Mayhany’s colleagues, however, have no such escape route.

As violence in Syria escalates and the regime increasingly targets academics, an international effort to support Syria’s beleaguered scholars with visas, fellowships, and guest appointments is gaining momentum. The Institute of International Education (IIE) in New York City has handed out 43 yearlong academic fellowships to displaced Syrians since the current conflict began. Now, it is appealing for funds, and for safe havens to step forward. Several hundred European universities have pledged to take at least one student, and the philanthropic Carnegie Corporation of New York has chipped in \$500,000 for fellowships, which provide up to \$25,000 to scholars. But “the need is 10 or 100 times what any of us are able to raise,” says IIE President Allan Goodman.

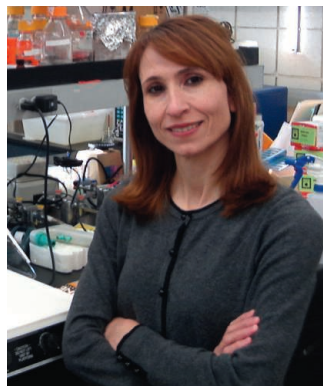
As Syria’s civil war drags into a third year, reports of interrogations and torture of professors are becoming commonplace. Dozens have been kidnapped for ransom or assassinated. University students are detained at checkpoints and conscripted to fight for the regime or for rebel groups.

About 30% of Syria’s professors have left the country, including many of the best, says Amal Alachkar, a neurobiologist from the University of Aleppo now at the University of California, Irvine. Some have ended up in refugee camps, while others have vanished. “Higher education, especially research, is collapsing,” she says.

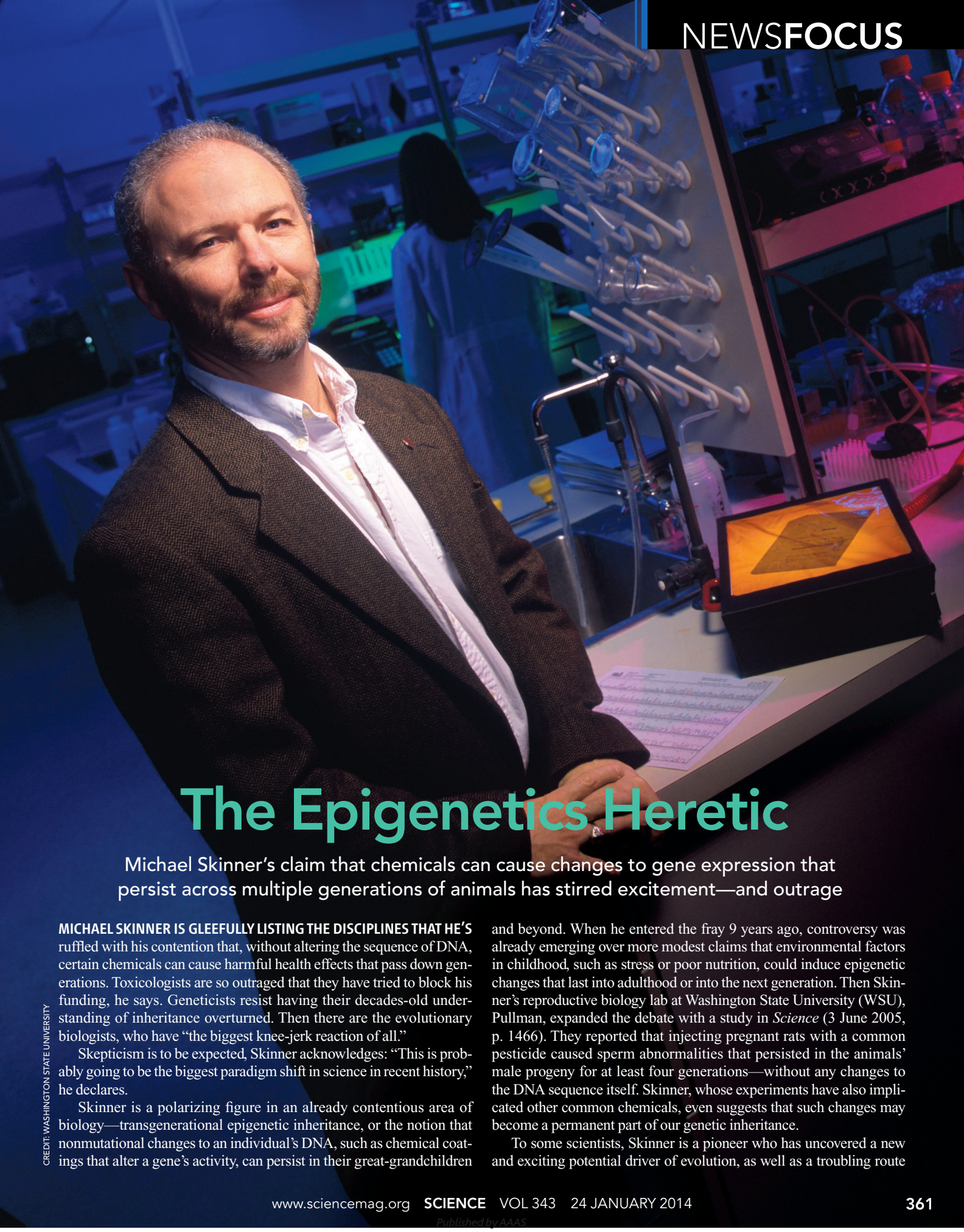
Conditions in Syria deteriorated much faster than they did in Iraq after the 2003 invasion, Goodman says. “What’s different about Syria is that universities were targeted right away, professors were threatened right away. The regime knew who their opponents were and instantly targeted them,” he says. “The immediacy of attacking education really hasn’t happened in any other place.”

In some ways, Syrian academia had thrived under President Bashar Assad, who took power following his father’s death in 2000. University enrollments rose and professors were encouraged to set up labs. But campuses have always been infested with security and intelligence agents, and “informers are everywhere,” Alachkar says. Regime loyalty, evidenced by membership in the ruling Ba’ath Party and overt patriotism, became the litmus test for faculty advancement, Alachkar says.

Students were taught to not question authority, but in the spring of 2011 they started to protest openly on many campuses. Alachkar says she encouraged her students to express themselves peacefully. “I



Safe haven. Amal Alachkar found succor at the University of California.



The Epigenetics Heretic

Michael Skinner's claim that chemicals can cause changes to gene expression that persist across multiple generations of animals has stirred excitement—and outrage

MICHAEL SKINNER IS GLEEFULLY LISTING THE DISCIPLINES THAT HE'S ruffled with his contention that, without altering the sequence of DNA, certain chemicals can cause harmful health effects that pass down generations. Toxicologists are so outraged that they have tried to block his funding, he says. Geneticists resist having their decades-old understanding of inheritance overturned. Then there are the evolutionary biologists, who have "the biggest knee-jerk reaction of all."

Skepticism is to be expected, Skinner acknowledges: "This is probably going to be the biggest paradigm shift in science in recent history," he declares.

Skinner is a polarizing figure in an already contentious area of biology—transgenerational epigenetic inheritance, or the notion that nonmutational changes to an individual's DNA, such as chemical coatings that alter a gene's activity, can persist in their great-grandchildren

and beyond. When he entered the fray 9 years ago, controversy was already emerging over more modest claims that environmental factors in childhood, such as stress or poor nutrition, could induce epigenetic changes that last into adulthood or into the next generation. Then Skinner's reproductive biology lab at Washington State University (WSU), Pullman, expanded the debate with a study in *Science* (3 June 2005, p. 1466). They reported that injecting pregnant rats with a common pesticide caused sperm abnormalities that persisted in the animals' male progeny for at least four generations—without any changes to the DNA sequence itself. Skinner, whose experiments have also implicated other common chemicals, even suggests that such changes may become a permanent part of our genetic inheritance.

To some scientists, Skinner is a pioneer who has uncovered a new and exciting potential driver of evolution, as well as a troubling route

CREDIT: WASHINGTON STATE UNIVERSITY

by which one generation's exposure to chemicals could contribute to diseases such as obesity and infertility in their descendants. "He's demonstrating that this occurs for a wide variety of chemicals. This was a big shockeroo" for industry, says psychobiologist David Crews of the University of Texas (UT), Austin.

But skeptics—and there are many—point out that Skinner's original experiments have not been replicated, despite several attempts. They find unconvincing his evidence that specific epigenetic changes to DNA are transferred through the germ line. "People will find it hard to believe until there are defined mechanisms," says reproductive biologist Cheryl Rosenfeld of the University of Missouri, Columbia.

Some are also put off by Skinner's uncompromising personality, which has contributed to upheaval within his university. "He's sometimes a little cavalier in the way he presents," says reproductive biologist John McCarrey, an old friend and sometime collaborator at UT San Antonio. "I think he feels like, 'I've shown these things and people aren't listening.'"

Man in black

Skinner seems to relish the role of maverick. He wears a suede Stetson and a long black coat during a recent interview in a downtown yogurt shop in Washington, D.C. He is in town to receive an "American Ingenuity" honor from *Smithsonian* magazine, awarded to 10 people who "are having a revolutionary effect" on their fields. A related profile in the magazine is the latest in a stream of favorable media articles recorded on Skinner's online curriculum vitae and lab website.

Skinner, whose family has deep roots in the Pacific Northwest, grew up on a ranch and started college on a wrestling scholarship. After earning a Ph.D. in biochemistry, he built a solid reputation as a reproductive biologist, studying the molecular biology of testes and ovary development and founding a center for reproductive biology at WSU with more than 100 faculty members.

His research took a turn around 2000 when a postdoc in his lab, Andrea Cupp, studied the insecticide methoxychlor, a so-called endocrine disruptor because it has hormonelike effects in the body. Cupp wondered whether the chemical would interfere with the formation of ovaries or testes in a pregnant rat's offspring if injected during a crucial window in fetal development. That did not happen, but as adults the male offspring had lower sperm counts and less motile sperm. By accident, Cupp bred these male offspring with the daughters of other pregnant rats that had been injected with the chemical. To her surprise, their male offspring—grandsons of the methoxychlor-treated pregnant rats—had the same sperm defects.

"I didn't believe her," Skinner says, because methoxychlor was not known to cause mutations that could account for the heritable effect. So he had Cupp repeat the experiment "about 15 times"—with the same result. Skinner's team saw the pattern again with another endocrine disruptor, the fungicide vinclozolin. Startlingly, the effects also showed up in subsequent generations of interbred rats, the so-called F₃ and F₄ generations.

The sperm problems were passed down to 90% of male offspring each generation, which suggested that some unexpected mutation could not be responsible. Mutations should be random and increasingly rare in each subsequent generation, Skinner says. Instead, Skinner's team identified a possible fingerprint of epigenetic changes in the rats' testes: methyl groups added to some genes, which could suppress their transcription into protein.

Although such methyl tags are known to pass down generations in plants and some other organisms, biologists didn't think this happened very often in mammals. That's because in the formation of sperm and eggs and in early embryos, cells go through a reprogramming stage believed to wipe away most methylation marks, except on a few genes crucial to early development. But the results from Skinner's team suggested that methylation marks on additional genes escape this reprogramming, even in generations that had no direct exposure to the toxin. (Skinner defines transgenerational effects as those in at least the F₃ generation, the great-grandchildren of the original animal. That is because treating a pregnant animal may also expose her embryos and the germ cells in those embryos to the toxin—see graphic.)

In their original study, Skinner's group did not hold back on the implications. "The ability of an environmental factor (for example, endocrine disruptor) to reprogram the germ line and to promote a transgenerational disease state has significant implications for evolutionary biology and disease etiology," they wrote.

The resulting *Science* paper became the most cited paper in reproductive biology for 2005; by now, it has more than 1200 citations, according to Google Scholar. But it also drew skepticism at toxicology meetings. Questions about the paper did lead to a lengthy clarification in 2010 explaining that key data from the original study

were not published in that paper but elsewhere. (Skinner says the data were omitted because of *Science*'s space constraints.)

More concerning to some, in three published papers, the latest last year, two labs at companies that make vinclozolin or a similar fungicide tried to replicate the vinclozolin rat experiment but found no effects beyond the first-generation offspring. An Environmental Protection Agency research group has reported similar results at meetings. "Doubt in the scientific community likely arises as a result of these

conflicting reports," Rosenfeld says.

Skinner says these studies were negative because they "didn't even come close" to following his protocol. In some cases, the researchers fed rats the chemical instead of injecting it, as he did. Or they used an inbred strain of rats instead of the outbred animals Skinner had studied. Toxicologists have long known that strains differ widely in their sensitivity to chemicals, he notes.

Some scientists attacked his work from behind the scenes, Skinner says. "I've had people try to get my [National Institutes of Health] grants revoked," he says. Others blocked further funding, he complains. He says that he has struggled recently to support his lab. "My funding has dramatically declined because we're pushing the envelope," he says.

Bumps in the road

Meanwhile, problems arose within Skinner's university. In 2008, he stepped down as director of WSU's Center for Reproductive Biology and later moved to another school within WSU because of what he labels "political battles" over a campus reorganization involving his center. Michael Griswold, dean of WSU's College of Sciences at the time, says he removed Skinner because the center needed a change in leadership after 12 years. Skinner also had "some disagreements" with members of his original school, Griswold adds.

Another shadow appeared on Skinner's professional record in 2010. Federal officials found that a Taiwanese postdoc in his lab had fabricated data in a 2006 *Endocrinology* paper. Skinner's group had retracted the paper in 2009 because they could not find some of the

"This is probably going to be the biggest paradigm shift in science in recent history."

underlying data. “I thought we had all the checks and balances in place, but clearly we didn’t,” Skinner says.

Yet Skinner has pressed ahead with his research. With Crews and his wife Andrea Gore, also at UT Austin, he reported in 2007 that when a female rat was caged with two different males—the F_3 male offspring of vinclozolin-treated pregnant rats and a control animal—the female shunned the male descended from a treated rat. The sperm abnormalities Skinner’s team had documented did not affect reproductive success, but this behavioral change could bias reproduction, suggesting that such multigenerational effects could play a role in evolution, Crews says.

Since then, Skinner has examined other chemicals, largely funded by a specific allocation—an earmark—within a Department of Defense (DOD) spending bill. His local congresswoman, Representative Cathy Rodgers (R-WA), and others earmarked \$3.7 million over 4 years to support his search for transgenerational effects from chemicals that soldiers might encounter. These studies, published over the past 2 years, showed that the insecticides DDT and permethrin, jet fuel, plastic additives known as phthalates and bisphenol A, and dioxin can all trigger transgenerational health effects in rats such as obesity and ovarian disease. Each resulted in a different pattern of methylation marks in the DNA of sperm, Skinner says. The DOD funding ended in 2011 when House Republicans banned earmarks.

Although his papers dominate the literature, Skinner notes that a handful of groups have also reported similar effects. For example, Emilie Rissman’s lab at the University of Virginia reported in 2012 that exposing pregnant mice to bisphenol A can cause changes in social behaviors and in behavior-related hormones, such as vasopressin, in their F_4 offspring. Several labs have suggested that diet and stress can also cause epigenetically controlled health effects that pass through to the F_3 generation. The weight of evidence has convinced some. “I’ve gone from skeptic to provisional believer,” says toxicologist Kim Boekelheide of Brown University.

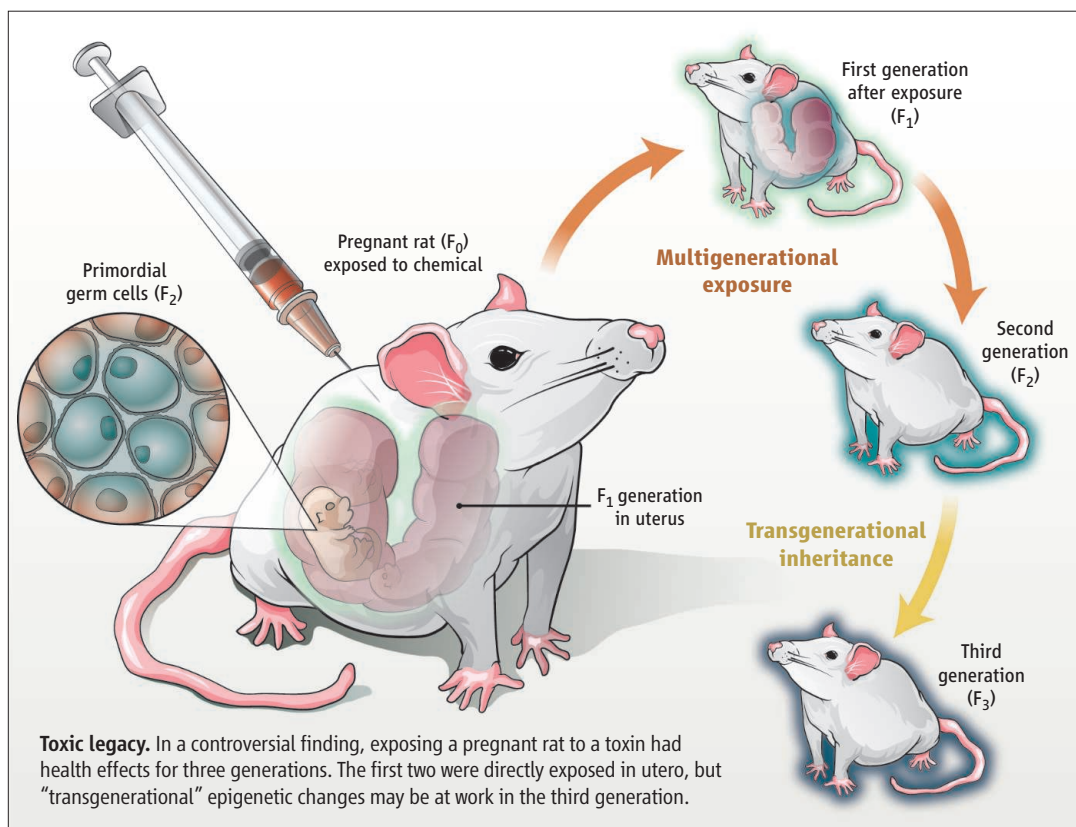
Lingering doubts

Some of the remaining skeptics speculate (often off the record) about factors other than epigenetic changes that could explain the effects Skinner’s lab has observed. For example, some kind of change in how the F_1 generation behaves or in the wombs of the chemical-exposed animals might alter the offspring’s health in a way that in turn influences the health of their descendants, says reproductive endocrinologist Richard Sharpe of the University of Edinburgh in the United Kingdom.

Skinner’s claim that he has identified permanent methylation changes in key genes, capable of resisting the normal erasure process in germ cells, has not won over the doubters. “I find the methylation differences unsatisfying,” says epigenetics researcher Oliver Rando of the University of Massachusetts Medical School in Worcester. He and others say methylation patterns vary widely among Skinner’s animals, so it’s hard to find a clear signal in the noise.

Skinner agrees that more data would help allay the controversy. If he can win funding, he wants to demonstrate that the specific methylation marks in a developing F_3 or F_4 generation male embryo match the methylation patterns in the adult animal’s sperm, which would support his claim that these marks are protected from the usual erasure process.

But he dismisses an approach that many have suggested could solidify his claims—that he artificially add methyl tags to spe-



cific genes to see if he can reproduce the effects he observes from exposures to pesticides and other chemicals. That’s impractical, he says, because his data suggest that “hundreds or thousands of epigenetic sites” are involved, and some affected genes may compensate for others. It is yet another example of the gulf between his views and those of geneticists, he says: They are “reductionists,” while “I am a systems biologist.”

To those who don’t flatly dismiss Skinner’s findings, he has raised a tantalizing glimpse of a new phenomenon, one that should be explored further. Transgenerational epigenetics “is either going to be blown away or it’s really going to be confirmed and expanded on and that’s what I find exciting” says epigenetics researcher Wolf Reik of the Babraham Institute in Cambridge, U.K.

Skinner doesn’t expect answers anytime soon. “I suspect that for the rest of my career, there will be skeptics,” he says.

—JOCELYN KAISER



Selling America's Fossil Record

Paleontologists fear that a growing commercial fossil industry is swallowing up U.S. fossils and the data they hold

Last November, in a marketing effort worthy of *Mad Men*, dinosaurs stood poised to take over Madison Avenue, courtesy of the New York auction house Bonhams. In a Manhattan atrium, giant mounted skeletons of *Triceratops* and *Tyrannosaurus rex* loomed over artfully arranged greenery, while a specimen of two individuals known as the Montana Dueling Dinosaurs—still partly encased in plaster field jackets—rested on black platforms. The venue looked just like a museum gallery, right down to explanatory placards. But the specimens were the property of commercial fossil hunters, dealers, and others looking to make a sale.

Nearly 20% of the specimens, ranging from a large Eocene turtle from Wyoming to an Oligocene false saber-toothed cat from South Dakota, were rare and carried auction estimates between \$100,000 and \$2 million. The star was the Montana Dueling

Dinosaurs, thought by some to contain both a new species of ceratopsid and a small tyrannosaur, and was projected to go for as much as \$9 million, a potential world record. Some thought the specimen might settle a scientific debate over whether a proposed small species, *Nanotyrannus lancensis*, is in fact just a juvenile *T. rex*. Thomas Lindgren, a co-director of Bonhams' natural history department, told *Science* that his role in organizing this auction was "probably the pinnacle" of his career.

But as curious New Yorkers wandered through the preview, many paleontologists fumed. To them, the specimens represent key data on the origins and evolution of species and on past environmental change. Few, if any, museums can pay the multimillion-dollar asking prices, leaving the market wide open to wealthy private collectors, says paleontologist Thomas Carr of Carthage

College in Kenosha, Wisconsin. "From what I've seen online, some of the specimens are scientifically significant, and it makes my blood boil that they are up for grabs," he says.

When auction day rolled around, most of the rare skeletons, including the Dueling Dinosaurs, failed to sell. The final bids were lower than the owners' minimum prices, suggesting a softening market for fossils. That was welcome news for paleontologists who oppose putting price tags of any kind on scientifically important specimens. But the high-profile auction exposed a growing schism in American paleontology, between those who sell fossils as merchandise and those who regard them as scientific data.

Commercial collectors say there are plenty of specimens to go around and that university-based paleontologists have access to fossils on millions of acres of U.S. public land, where private collectors usually can't

CREDIT: ANTHONY BEHAR/SIPA USA/AP PHOTO

On the block. A New York auction house displays fossils for sale, including ancient shark teeth mounted in a reconstructed jaw.

get excavation permits. “If they don’t like the free enterprise system, don’t participate in it, but don’t demonize those that do,” argued Mike Triebold, president of Triebold Paleontology Inc. in Woodland Park, Colorado, in an e-mail interview.

But paleontologists say that most fossil vertebrates and some plants and invertebrates are rare and deserve scientific study, wherever they are found. “If you find an Egyptian tomb, do you put the contents in a museum, or do you sell them?” asks paleontologist Catherine Forster of George Washington University in Washington, D.C., president of the Society of Vertebrate Paleontology (SVP). “At one time people sold Egyptian mummies to tourists, and I see the same thing happening now to fossils.”

As a result, valuable information is vanishing, say many paleontologists. “A lot of bridges have been burned between the commercial collectors and the academic world,” says paleontologist Stephen Brusatte of the University of Edinburgh in the United Kingdom, who studies ancient mammals from New Mexico. “A lot of my colleagues have had enough.”

The good old days

Commercial collectors once worked hand in hand with academic paleontologists. In the early 19th century, for example, British fossil dealer Mary Anning discussed the anatomy of fossil fishes with famed Swiss biologist Louis Agassiz and discovered key ichthyosaur and plesiosaur specimens that helped clinch the idea that some species had gone extinct.

Later, as museums and educational institutes started amassing large fossil collections, they dispatched private collectors into the field. One famous American fossil hunter, Charles Sternberg, hunted specimens in western North America for nearly a dozen museums and universities between the 1870s and the 1920s. In 1921 alone, Sternberg collected 113 fossil specimens, including one complete dinosaur, for Uppsala University in Sweden. He got paid \$2500, according to a 1992 paper put out by the New Mexico Geological Society.

Now, these collegial relations have soured in many quarters, creating an angry atmosphere that some compare to the biting partisan relations between political parties in the U.S. Congress. Over the past 20 years, the number of commercial companies involved in the fossil trade in

the United States has doubled, from about 75 companies and individuals to about 150 today, estimates paleontologist Peter Larson of the Black Hills Institute of Geological Research Inc. in Hill City, South Dakota; in 1977 Larson helped found the Association of Applied Paleontological Sciences (AAPS), an organization of commercial collectors, dealers, and paleontologists.

Under U.S. law, amateur and professional fossil hunters can collect specimens on private land—with the landowner’s permission—and sell them to whomever they choose. In exchange for an exclusive license to collect, fossil hunters may offer a percentage of sale proceeds to landowners, often ranchers in the western United States. A sample license published online by AAPS specifies a 10% cut for the landowner. That’s a tempting incentive in dinosaur-rich regions like Garfield County, Montana, where the median household income between 2007 and 2011 was \$37,500, 29% less than the U.S. median. “Many ranchers need opportunities to make money off their resources, and dinosaur fossils are just one more resource that they

Sues, curator of vertebrate paleontology at the Smithsonian Institution’s National Museum of Natural History in Washington, D.C. For example, paleontologists from one New Mexico museum say they spent several seasons excavating important semiaquatic reptiles from Triassic rocks on a private ranch, when they were suddenly denied access to the land: A commercial fossil hunter had swooped in and purchased an exclusive collecting right.

Early fossil hunters sold their impressive finds largely to museums, but today many collectors seek private buyers. Near Fossil Butte National Monument in Wyoming, for example, collectors work the Green River Formation, layers of fine-grained lake sediments dating to some 50 million years ago, to find beautiful fossils of Eocene fish, turtles, and other species. The website of one company, GeoDÉcor, (accessed at www.geodecor.com on 6 December 2014), where Lindgren is chief executive officer, sheds light on how the fossils are marketed. After workers quarry fossil specimens by hand, conservators in a GeoDÉcor laboratory



Ready to sell. Montana collector Clayton Phipps hoped his specimen with two dinosaurs would fetch \$7 million or more at auction—a price too high for most museums.

can potentially get money from,” says paleontologist Gregory Liggett of the Bureau of Land Management in Billings, Montana.

These private licenses can be serious obstacles to research, some paleontologists say. Unlike commercial collectors, academic paleontologists have no sales proceeds to divvy up with landowners. “With this overheated market [for fossils], landowners are barring paleontologists from working on their land, because now all they are seeing is dollar signs,” says Hans-Dieter

prepare fossil-strewn slabs as tiles or wall murals to be sold as decorations. The GeoDÉcor website shows these specimens in upscale living rooms, kitchens, and bedrooms.

Buyers need not be fossil enthusiasts. “We have targeted people who like nature, people who are into hunting and fishing and boating,” says Greg Laco, president of the Logan, Utah-based Green River Stone Company, which specializes in selling Green River fish tiles and murals. “We have kind of broadened that market.”

Forster deplores the interior design trend, saying consumers may end up ripping out the fossils and throwing them away when they remodel their homes. “It’s just a complete waste,” she says. “I wish people would see them as beautiful pieces of data rather than something they need to possess.”

In search of the dawn horse

Many Green River fish species are well known, so not every fish specimen from the formation is scientifically important. But sometimes collectors find more than fish. In the early 2000s, for example, Jim Tynsky of Tynsky’s Fossil Fish Inc. in Kemmerer, Wyoming, collected a beautifully preserved small horse from the Green River Formation. He allowed researchers from the Field Museum in Chicago, Illinois, to photograph and make

four casts of the specimen, and paleontologist Aaron Wood of the Florida Museum of Natural History in Gainesville tentatively identified it as *Protorohippus venticolus* from a photograph. This specimen, the most complete skeleton of a dawn horse ever discovered, is of “great scientific importance,” Sues says. The Equidae, or true horses, first appeared in North America about 55 million years ago, and “the earliest horses are primarily represented by jaws and teeth,” he explains. “Even partial skeletons are of great interest because they provide insights on locomotion and other aspects of biology.”

The original specimen is still in private hands, where it cannot be readily studied by scientists. “I approached several museums about [buying] it,” Tynsky says, “but never came to an agreement about pricing.” In December, the specimen was listed online for sale by Touchstone Gallery of Cedar Crest, New Mexico. According to a gallery representative, the asking price is now \$2.25 million.

Triebold says that the high prices of large fossil vertebrates are warranted. To excavate and prepare an average elephant-sized dinosaur skeleton, for example, can take between 15,000 and 20,000 hours. “A million dollar price tag on a dinosaur doesn’t mean you have made a million dollars,” writes Triebold in an e-mail. “You might even have lost some money if you aren’t careful.”

Paleontologist Jack Horner at the Museum of the Rockies in Bozeman, Montana, counters that he and his colleagues collected

nearly a dozen *T. rex* and 100 *Triceratops* specimens at a total cost of \$2 million during their recent Hell Creek Project. Based on this fieldwork, he and his students have published 47 peer-reviewed papers since 1999, including three in *Science* (25 March 2005, p. 1952; 13 April 2007, p. 277; and 3 June 2005, p. 1456), as well as one edited volume and eight dissertation theses.

Museums do often buy from commercial collectors, if they can find the funds. SVP’s ethical code censures the “barter, sale or purchase of scientifically significant vertebrate fossils,” but makes an exception for accredited museums that will conserve and study specimens. In November, for example, the Natural History Museum of Denmark in Copenhagen used funds from a private foundation to buy a complete skeleton of *Diplodocus longus* at an auction in Sussex, U.K. Excavated in Wyoming, the specimen sold for \$789,205.

But the high prices are driving many museums out of the market. Carr, a senior scientific adviser for the Dinosaur Discovery Museum in Kenosha, and others think that the trend toward multimillion-dollar price tags began in 1997, when the Field Museum outbid other buyers at an auction for a nearly complete *T. Rex* specimen—the dinosaur called Sue, now displayed in the museum (*Science*, 19 September 1997, p. 1767). By combining forces with corporate donors, the museum paid \$8,362,500 for the specimen, a world record. After this, Carr says, many collectors upped prices, convinced that museums would find a way to raise the money.

Sue’s sale price became a benchmark, agrees vertebrate paleontologist Peter Makovicky of the Field Museum. Although “no one has ever hit that benchmark again, a lot of people dream about it,” he says. He calls the trend toward higher prices “a bit unfortunate,” but says the situation isn’t much different from the art market, for example when art sold at exceptionally

Fossil riches. Two geological formations in the western United States (see map, left) have produced spectacular fossils, from rarities like Sue and a dawn horse to common specimens like *Knightia*.

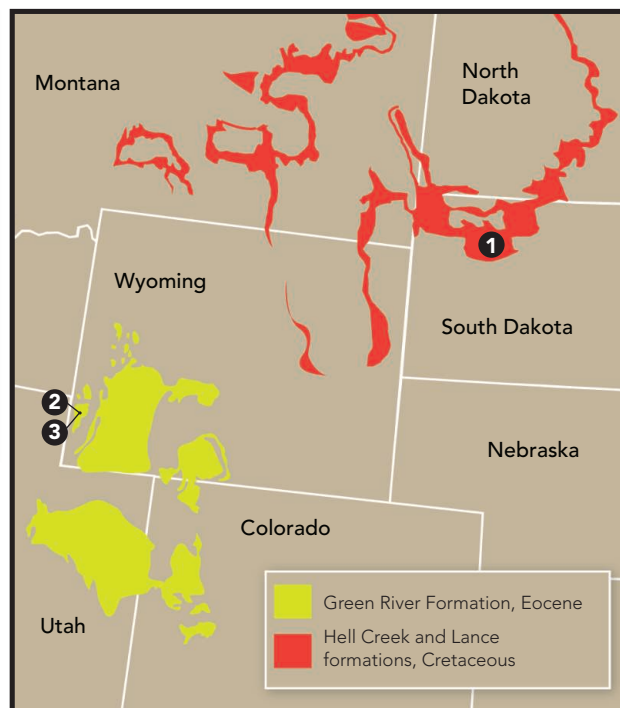
1 The *T. rex* called Sue



2 *Protorohippus*, a type of dawn horse



3 *Knightia*, an Eocene fish



high prices during the 1990s.

But the financial crisis of 2008 badly dented public funding for museums and also made it harder to raise private money. Today, many American museums are struggling: the Carnegie Museum of Natural History in Pittsburgh announced in December that it is losing as much as \$2 million a year, and the Smithsonian Institution faced a \$65 million budget cut last year. The San Diego Natural History Museum in California even planned to sell fossils to raise money to buy other natural history specimens. Although museum CEO Michael Hager told *Science* that the museum is “in great financial shape,” it put up several lots of fossils, including some specimens included in published papers, for the recent Bonhams auction. After SVP protested the move and other public institutions offered to take on the fossils, the museum withdrew the lots.

Finances aren't the only barrier to museums buying at commercial auctions. Horner argues that many commercial collectors leave behind vital contextual information, such as stratigraphic data and sedimentary clues to how animals were preserved after death. This makes the specimens less scientifically valuable. “There is a variety of data that they might collect, but every bit they do is overhead, so there is no possible way that they are going to get as much as we do,” Horner says.

Professional collectors dismiss these criticisms. They say they have far more experience collecting specimens, and so do a better job, than paleontologists who spend most of their time teaching or working in museums. And some commercial collectors publish scientific papers on their finds.

Horner notes, however, that commercial collectors of dinosaurs tend to invest an inordinate amount of time and resources on reconstructing specimens—something that he and his colleagues at the Museum of the Rockies do not do. The *Triceratops* skeleton consigned to Bonhams in November, for example, was only about 65% original bone. According to the auction catalog, preparators used casts of missing bones to complete the skeleton. Such restoration may improve a specimen's appearance, but can impede research, Horner says. When museums

purchase such specimens, “the scientists who get it have to scrape on it to see what's plaster and what isn't,” he says.

The public-private divide

Both commercial and research paleontologists say they are dissatisfied with the current system. Commercial collectors complain bitterly about the Paleontological Resources Preservation Act, passed in 2009,

permit, generally issued only to qualified paleontologists. The legislation, passed in 1978, has “curtailed the excavation by untrained individuals resulting in damage and scientific loss of specimens,” says Dan Spivak of the Royal Tyrrell Museum in Drumheller, Canada.

But property rights are considered sacrosanct in much of the American West, and similar legislation would almost certainly not

pass in the United States. So Carr recently suggested in his blog a different tactic: to apply the “eminent domain” clause in the U.S. Constitution to important dinosaur fossils. This clause permits federal and state governments to seize land for public use such as building a highway, provided that landowners are compensated. “If the federal government could be convinced that [scientifically important] fossils fall under the definition of ‘land,’ ... then possibly they could be seized and the people who collected the specimens could be compensated for the expense of collection, salaries, and whatever monetary value you place on fossils,” Carr says.

The idea already has sparked a strong backlash from collectors. “Telling a third-generation rancher that he doesn't or shouldn't own the dinosaur that was collected on his property or what he should do with it, would be a conversation that would almost certainly enlighten the scientist on the ways of America in a big way,” Triebold says. “You don't mess with private property rights in ranchland.”

Given the war of words and fraying tempers, some paleontologists advocate stepping back and working to rebuild the old cooperative relationships with commercial collectors. Rather than asking the government to seize fossils from collectors, Forster says, “I would rather see academic paleontologists work with commercial collectors to parse significant from non-significant [fossils], and work to keep them in the public domain” by providing advice and contacts with potential museum buyers. To Forster, finding such a solution is urgent. “We are losing vast amounts of information about our collective history,” she says. “It's disappearing for no particularly good reason, and it's not coming back. Gone is gone.”

—HEATHER PRINGLE

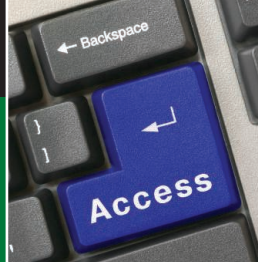


Backsplash. Slabs with 50-million-year-old fossil fish decorate kitchens and bedrooms.

which restricts collection of scientifically important fossil specimens on federal land to qualified researchers with a permit. Permit holders must deposit all collected fossils in a museum or other approved repository—no sales allowed. According to 1995 National Wilderness Institute data, federal lands make up 26% of the U.S. land mass, but the figure varies widely by state. Nearly 81% of the state of Nevada is public land, for example; in Texas, it is just 1.4%.

Triebold, a past president of AAPS, thinks Congress should have instituted a system that gives commercial collectors permits to operate on federal land in exchange for royalties on sales. Instead, he writes in an e-mail, “the public lands of the U.S. have officially become the private sandbox for only those institutional collectors who are ‘qualified.’”

In contrast, Carr and other academic paleontologists would like to see protection taken further, to private land. Many other governments have already taken this step. In the Canadian province of Alberta, which has rich fossil beds, all specimens, whether found on public or private land, belong to the government. Excavation requires a

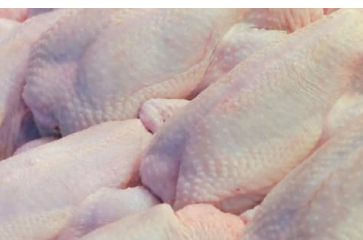


LETTERS

edited by Jennifer Sills

Flu Threat Spurs Culture Change

IN THE NEWS & ANALYSIS STORY "TENSE VIGIL IN CHINA AS NASTY FLU virus stirs back to life" (C. Larson, 29 November 2013, p. 1031), George Gao, the deputy director-general of the Chinese Center for Disease Control and Prevention, is quoted as saying that "You cannot change culture," suggesting that there is no point in trying to change the live poultry markets in China. Evidence contradicts this assumption.



During the first A(H5N1) influenza outbreak in Hong Kong in 1997, prompt government action regarding live poultry markets and culling cut the epidemic short. After repeated reemergence of A(H5N1) in the first decade of the century, the Hong Kong government first proposed a central slaughterhouse to remove live poultry from wet markets, but trade and NIMBY resi-

dential opposition deferred that decision indefinitely (1). Our initial population surveys indicated that individuals were more willing to change their habits of buying if such changes were observed among others in the community (2). The population pragmatically realized that a change in the practice of poultry retail was needed.

The Hong Kong government subsequently initiated a policy of reducing live poultry availability through reduced imports and internal production, as well as buying back licenses from traders who sell live poultry for slaughter, so that today such stalls are a rarity. Instead, chilled poultry is widely available, with many former live poultry stalls now selling pre-killed and chilled poultry. Population exposure to live poultry from markets declined by 60% between 2004 and 2006 and by a further 21% between 2006 and 2010, attributable to reduced live poultry availability and public health education (3, 4).

Thus, culture can be changed. We believe that cultural changes that promote public health principles of separating the agent, vector, and host are China's only real long-term solution to repeated influenza emergence.

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Regulating Dual-Use Research in Europe

THE EUROPEAN SOCIETY FOR VIROLOGY (ESV), which represents scientists active in basic, medical, veterinary, plant, and environmental aspects of virology, has been following with particular interest the debate on "gain-of-function" studies catalyzed by the

H5N1 influenza virus transmission studies from the Dutch group led by Ron Fouchier and published by *Science* in June 2012 (1). The Dutch government insisted that Fouchier ask for official permission (an export permit) before publishing his research abroad, on the basis of European Council regulation EC 428/2009, which was issued in 2009 to prevent the spread of nuclear, chemical, and biological weapons. Although Fouchier complied before the *Science* paper was published, Erasmus MC Rotterdam sued the government over the requirement for an export permit, based on the fact that the Annex to Council regulation EC 428/2009 contains an exclusion clause for basic scientific research and information already in the public domain. The appeal was recently rejected by a district court, and the case is now being brought before Amsterdam's Court of Appeal.

The News of the Week story "Virologist

appeals decision on H5N1 export rules" (8 November 2013, p. 676) stated that the ESV has "sided with Fouchier." Roughly 90 viruses and microorganisms that can cause disease in humans, animals, and plants are listed in the Annex to Council regulation EC 428/2009. ESV's position stems from the concern that results from scientific work carried out in Europe on these organisms would require an export permit before they can be published in international scientific journals.

This prospect raises a number of serious issues. Under what circumstances should this EC regulation be applied to biomedical research? Who is going to decide when the EC regulation does or does not apply? What should be considered "basic scientific research," and who is going to judge this criterion? (This is not a trivial question, especially in the European Union context, where, in theory, there might be 28 different inter-

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

pretations of the same regulation.) Does this create the potential for discrimination among scientists working in different European States and between European scientists and those in the rest of the world? Does this decision apply only when specific results are going to be published in journals outside Europe, or does it apply universally?

It may be that controversial questions related to this issue were ignored for too long, allowing a precedent to be set prematurely. We are overdue for discussions on how to regulate the dissemination of “sensitive” data in a way that does not compromise biosecurity, while maintaining the principle that acquiring important and meaningful knowledge cannot simply be stopped. ESV believes that export control does not represent the best way to deal with this issue.

Our intention is not to criticize or to disregard the work of jurisprudence experts. We believe that the European Commission should take steps to promote a common understanding of the current regulation by existing working groups or by a new advisory committee created to deal with the dual-use research in a harmonized and balanced way throughout

Europe. In the meantime, we have expressed our willingness to provide law officers with proper scientific advice, making available the expertise of our many European scientists.

GIORGIO PALÙ

President of the European Society for Virology, Department of Molecular Medicine, University of Padova, 35121, Italy. E-mail: giorgio.palu@unipd.it

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Misleading Results: Translational Challenges

WE AGREE COMPLETELY WITH THE NEWS FOCUS story “When mice mislead” (J. Couzin-Frankel, 22 November 2013, p. 922). Many of the issues identified in this article, such as investigator bias, blinding, randomization, and inclusion and exclusion criteria, were clearly identified in the Stroke Therapy Academic Industry Round Table publication in 1999 (1) and discussed further by others (2). Tightening the standards for animal experimentation, especially for those experiments that test drugs for their neuroprotective

effects, is critical as we try to translate these drugs into human stroke therapy.

There have been hundreds of drugs tested for neuroprotective effects and many, if not most, have positive effects, in animal models. If a mouse or rat had a stroke, we would know exactly how to treat it. Unfortunately, none of these drugs has shown success in humans. It is unlikely that poor methods used in animal studies account for all the negative clinical trials that have been performed based on preclinical studies. After all, some investigators do perform appropriate experiments, and even those studies rarely lead to positive clinical trials.

One important issue not mentioned in the News Focus piece could also account for the lack of positive clinical trials: Most researchers in the stroke field work with normal, young, healthy animals. Humans who participate in neuroprotective drug clinical trials are most often not normal, young, or healthy. They have a variety of comorbid diseases such as hypertension, diabetes, obesity, and vascular disease, which could alter the way in which drugs work in humans. We suggest that in future animal experiments, drugs be tested in

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animals that are aged, with comorbid diseases. Such studies may help to identify potentially effective drugs in an animal model that mimics more closely a human with stroke.

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Misleading Results: Don't Blame the Mice

IN THE NEWS FOCUS STORY "WHEN MICE MISLEAD" (22 November 2013, p. 922), J. Couzin-Frankel raises concerns about practices that give rise to misleading scientific findings. The case studies cited demonstrate procedural

errors that can occur in the design or execution of experiments. These unfortunate mistakes can happen in any area of research and are not the exclusive domain of mice or animal models; clinical research is certainly not immune to error. Even well-executed studies can give rise to misleading results for reasons that are insidious and hard to control (1).

With regard to mice, the first "risk of bias question" that a reviewer should ask is, "Was the result replicated in more than one genetic background?" Idiosyncrasies of particular mouse strains can be misleading (2). Ignoring the effect of genetic background is the most pervasive source of bias in animal studies.

To reduce the problem of false or irreproducible scientific findings, we need to address two root causes. Science today is driven by an incentive system that often rewards precedence and impact over quality of the work. Statistical training of scientists often emphasizes analytical techniques over experimental design and quantitative reasoning. These are systemic problems that will not change without substantial effort. However, the message in this News Focus story does suggest some simple advice that

can redress many of the pitfalls of experimental studies: Be wise, randomize.

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CORRECTIONS AND CLARIFICATIONS

Table of Contents: (3 January, p. 3). The artist's name was misspelled in the cover caption credit line. The correct spelling is Aurore Simonnet. Additionally, the Review Summary by M. Dalziel *et al.* was mislabeled as a Research Article Summary, and the URL for the full text was incorrect. The correct URL is <http://dx.doi.org/10.1126/science.1235681>. The HTML and PDF versions online are correct. The title of the Perspective by Y. E. Türkmen and V. K. Aggarwal was also incorrect. It should be "A Simpler Route for Making Nitrogen-Alkene Rings." The HTML version is correct online, and the PDF version has been corrected.

Editorial: "No windfall for U.S. science" by M. McNutt (3 January, p. 6). In the second paragraph, the U.S. Senate budget mark is \$1,058B, not \$1.058B. The HTML and PDF versions online have been corrected.

Essay: "Science & SciLifeLab Prize" (6 December 2013, p. 1185). Finalists have been reclassified as Second Runners-Up. The HTML and PDF versions online have been corrected.

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CLIMATE CHANGE

An Economist's Journey

Mark Jaccard

For over two decades, Bill Nordhaus of Yale University has been recognized as a leading climate economist, and for much of that time his work has provoked the scientist-versus-economist debate in my graduate seminar on sustainable energy. Lately, however, he has begun to disappoint—as demonstrated by *The Climate Casino*.

In the scientist-economist debate, the plea of scientists is straightforward: Don't mess with Earth's complicated systems by loading the atmosphere with greenhouse gases, notably carbon dioxide (CO₂) from burning coal, oil, and gas. And don't allow global temperatures to rise more than 2°C because this will significantly increase the likelihood of harmful surprises, like accelerated global warming as melting permafrost releases methane (itself a potent greenhouse gas).

Economists, in contrast, argue that because humans also benefit from burning fossil fuels, CO₂ reduction has benefits and costs, and both must be weighed in finding “the optimal path of carbon pollution”—a wonderfully provocative phrase in my seminar as elsewhere. But this cost-benefit analysis of economists is tricky: It requires monetary values for everything we care about, not just the goods and services recorded in gross domestic product. We need the dollar values of the 2000th-to-last polar bear and the 200th-to-last (which are not the same), of the lost cultural heritage if Venice succumbs to rising seas, and of reducing the chances of harmful surprises like methane releases.

Undaunted, the intrepid Nordhaus built a model that integrates Earth systems and the global economy in calculating the optimal path of carbon pollution. In a widely cited paper (1), he concluded that we should allow rapid CO₂ increases even though the temperature would climb more than 3°C by 2100, momentum carrying it beyond 4°C in the next century. In essence, the economic growth benefits from burning fossil fuels outweigh the costs from the resulting climate change. Although Nordhaus proposed a small, rising tax on carbon, his analysis essentially vindicated business as usual for our fossil fuel-addicted economy.

Needless to say, his work attracted criti-

cism from scientists and some economists. They argued that he undervalued harms to the natural world, was overly optimistic that economic growth could compensate future generations for the vicissitudes of a hotter climate, and downplayed the risk of catastrophic outcomes.

Climate Casino's cost-benefit analysis suggests that these critiques had some effect; Nordhaus's numbers have changed. But the book is much more than an update. It provides an exceptionally clear and balanced primer on climate science, economics, and policy.

It's a must-read for natural scientists and social scientists, climate skeptics and climate hawks, Democrats and Republicans. My only disappointment is that Nordhaus now suggests we limit the temperature increase to between 2° and 3°C—too close to the cap favored by scientists to provoke my students.

In some respects, Nordhaus has not changed. He still rejects arguments that we should weigh future costs and benefits equally with present ones. Like most economists, he believes that we should discount those future values when comparing to the present because economic growth ensures that future people will be richer. Decades of rising wealth and technological innovation

will enable them to better deal with climate change. This is a major reason why his optimal path includes a somewhat hotter world than the 2°C favored by scientists.

Yet his optimal path is now much closer to that of scientists because of his heightened concern with 3°C as “the danger zone for several important tipping elements.” These include accelerating methane release, sudden collapse of ice sheets such as the West Antarctic, and large-scale changes in ocean currents such as the Gulf Stream. Inclusion of these and other risks leads his model to exhibit rapidly rising damages beyond 3°C.

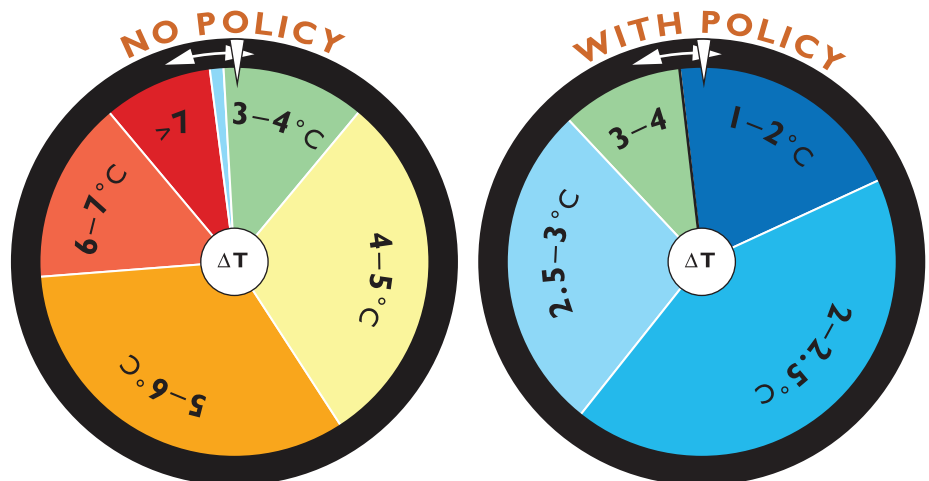
Scientists will now be less unhappy with Nordhaus, but I doubt they'll be completely happy. As a hard-nosed economist, he shows that much of our economy, including food production, is highly managed and thus able to handle modest global warming, perhaps even benefit from it. Some scientists don't want to hear this.

In addition, Nordhaus seems to suggest that his model undervalues the harms for unmanaged ecosystems as global warming and ocean acidification intensify. Economists have long tried to estimate these values, but their methods often involve hypothetical questions such as “What would you pay to save the polar bears?” Like many economists, Nordhaus finds the answers “too unreliable at present to be used for assessing the costs of ecosystem effects triggered by rising CO₂ concentrations and climate change.”

So what does he use instead? This is the

The Climate Casino
Risk, Uncertainty,
and Economics for
a Warming World

by William Nordhaus
Yale University Press, New
Haven, CT, 2013. 392 pp. \$30,
£20. ISBN 9780300189773.



Wheels of chance. The Greenhouse Gamble™ wheels, developed by the MIT Joint Program on the Science and Policy of Global Change, depict the likelihood of an increase in global average surface temperature between 1990 and 2100. With no actions taken to curb the global emissions of greenhouse gases, there are even odds of that falling above or below 5.2°C. If policy changes limit the century's cumulative emissions of greenhouse gases to 4.2 trillion metric tons, the median predicted warming level is 2.3°C (2).

one place in an otherwise transparent book where I couldn't find a clear answer. Nordhaus says we need more research and that the value should not be zero. But he doesn't explain how he chose his values. I was left with the impression that those polar bears don't get a fair hearing.

And I was surprised that Nordhaus doesn't discuss the approach to valuing the environment long used in electric utility regulation. In assessing generation options, utility economists and planners show a representative sample of customers the increases in their

electricity bills resulting from energy supply choices that reduce certain environmental harms. If people accept such increases, we can calculate a minimum willingness to pay for protecting these environmental amenities. Extending this "abatement cost" approach to climate change is not easy, but scientists and many others would certainly prefer doing so to knowingly undervaluing the environment.

As Nordhaus knows, those wealthier people in 2050 and 2100 will have different preferences. The desire to preserve as much as possible of the natural world is a luxury

good (which increases with income). Thus, we might assume that future generations will wish we had allowed a slightly slower economic growth (losing a few years of conventional growth according to Nordhaus) in order to preserve at least some of the ecosystems now threatened by global warming.

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10.1126/science.1248487

COMPUTERS AND SOCIETY

Bordering Fiction

Albert-László Barabási

What is absorbing about Dave Eggers's latest novel is not the dystopic world it depicts but the way that arose: Everyone at The Circle, a Google-Amazon-Facebook-Twitter mash-up, is eager to make the world a better place. Engineers at heart, they relentlessly innovate to reduce crime, to organize and store all information, and to leave no one behind. While their motivations are pure, each of their products is a subtle slide toward the 21st century's version of Orwell's *1984*—not a world in which a selected few control many but one where everyone monitors everybody. As the company helps us become "all-seeing, all knowing," privacy becomes a crime, and the upbeat culture of The Circle morphs into an organization whose core values are lifted from the playbook of the U.S. National Security Agency (NSA): "Privacy is theft." "Secrets are lies."

A few days into her dream job at The Circle, Mae Holland learns that pleasing consumers forms only a tiny part of her responsibilities. The company demands never-ceasing engagement—an expectation that she fully immerse herself in the wondrous activities the organization offers its employees. She must mingle with politicians, applaud singers, and praise the cooking of celebrity chefs. Most important,

she must share all her experiences online. Absenteeism is a sign of detachment. Not sharing is a crime.

Early in the novel, a potential love interest uses Mae's profile to demonstrate an application that packages all online data about her to provide the lowdown for a first date. Mae is outraged as she watches her food allergies and favorite dishes paraded on the big screen. Seemingly, a relationship turned sour before it could really begin. In reality, an episode that defines Mae's trajectory, allowing

us to witness her profound transformation over the next 300 pages. Responding to the culture of her new workplace, Mae gives up a little more of her privacy each day. Eventually she becomes the powerful official poster girl of The Circle's open-book philosophy. Her life, with minute resolution, is on display for everyone to witness.

Eggers in *The Circle* rides a wave that has been brewing for years now. I have argued that given the high predictability of human activity (1, 2), services like the novel's fictitious SeaChange (a vast array of cameras that monitors everyone everywhere) make not only our present but also our future increasingly transparent to the highest bidder (3). Fiction beats nonfiction, however, in its ability to portray the individual motivations—or the lack of them—of the devel-

opers that nudge us toward an increasingly transparent society. Eggers's page turner works because it requires no implausible breakthroughs. Its familiarity gets under our skin, as Eggers offers a chilling image not of a distant world but rather of one that feels eerily everyday.

To be sure, some of the plot elements border on incredibility. The United States would never hand over voting and social security to a private company like The Circle. Law-

makers would never agree to the lack of privacy The Circle's technologies perpetuate. These are alarmist twists that work only in fiction. But are they? For years, I was told that U.S. laws forbid federal access to my mobile phone records. Then Edward Snowden revealed that NSA did in fact strong-arm that

data away from the carriers, jolting me into abandoning my research on anonymized phone records altogether. I also argued that the U.S. government lacks the personnel and know-how to build the sophisticated tools it dreams of using (3). Indeed, some of our best students and colleagues flocked to Facebook, Google, Twitter, and Amazon; I know of no one who chose NSA. Then Snowden revealed that NSA simply purchased the know-how from the Valley. I have thus stopped believing that there is a wall between reality and fairy tales. So I read *The Circle* not as science fiction but as a case study of a world in which we currently live: a stress test that reboots *1984* for the digital age.

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10.1126/science.1248660



The Circle A Novel

by Dave Eggers

Knopf, New York/McSweeney's Books, San Francisco, 2013. 501 pp. \$27.95. ISBN 9780385351393. Hamish Hamilton, London. £18.99. ISBN 9780241146484.

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INFORMATION ACCESS

Raw Personal Data: Providing Access

Jeantine E. Lunshof,^{1,2*} George M. Church,¹ Barbara Prainsack³

Heated debates on responsibilities in biomedical research currently focus on the end of the data and information pipeline: They revolve around issues of returning results to participants and patients (1–3, 4). Although these debates are timely, they miss a crucial point at the beginning of the pipeline: the question of whether sample donors are able to access the raw data derived directly from their stored sample. The U.S. Presidential Commission recently reviewed 32 reports from the United States and worldwide on returning of findings in diverse contexts (4); it is striking that access to raw data by participants was not addressed in any of them.

In this Policy Forum, we argue, first, that there are compelling ethical reasons for enabling donors to access the raw data derived from their material deposited in any kind of repository; second, that there is a crucial difference between providing access to data and returning findings.

A One-Way Transaction

Drawing an analogy between data banks and money banks, despite their differences in other respects, may be instructive to illustrate the crucial point of data access. In everyday life, banking is a two-way interaction. When we make a monetary deposit to a bank, we get a receipt. Furthermore, we are able to access our account and see the balance, we can track transactions, and we can terminate our relationship with the bank and have the remaining balance returned. As an account holder, we can directly access our own data.

When we make a deposit to a data bank or biorepository, in contrast, putting our data or specimen into the hands of researchers or clinicians, we lose track of it. We do not have the status of account holders. We are not even able to access the basic descriptors of our own



contribution: The raw data, directly from the deposited sample before analysis, are—with very few exceptions (5)—not made available to the depositor. Thus, a deposit to a research or clinical repository is a one-way transaction in which we give up most of our agency and control.

Data are shared and used by a large number of researchers; the individual donors are those with the least access to the data they contributed. This one-way communication is a blatant anachronism in the relationship between data donors and researchers or clinicians, and unnecessary when relatively low cost, user-friendly Web-based tools to facilitate such access are available. Today, samples and data sets are usually identified and tracked through bar codes. Handing over that bar code to any research participant or patient when the sample or the survey is taken can enable access if the system is set up for that. It is then the individual who decides whether to use the access code and look at her or his raw data.

Getting It Right from the Start

Web-based information and communication technologies render direct data access technically feasible and economically affordable in many cases. But repositories have to be designed accordingly right from the start, as later adaptation will often be expensive and difficult. Data storage formats need to be interoperable in order to build a framework

Donors should have access to raw data derived from their contributions to research or clinical repositories to increase personal choice and reciprocity.

for data sharing, a requirement that may be very challenging in a competitive setting with diverse research and commercial interests. Databases that provide public open access, as, e.g., the Personal Genome Project (5), avoid such problems, and for them, issues of donor access are moot. But also studies for which public access is not an option should pay urgent attention to the structures and conditions necessary to provide donors access to their raw data.

In actual practice, the financial and logistic challenges related to providing access might result in significant additional costs, costs that participants could decide they do not want to incur (6). But data access needs to be an option, in order to enable a personal choice.

A Fair and Reciprocal Relationship

Although we use biorepositories as an example to illustrate our argument for reciprocity and fairness, the argument is applicable to any field where researchers and participants interact. Participants' access to raw data could be a helpful mechanism to increase transparency in any study, be it genomics, other "omics," or social psychology. Data fabrication would be much more difficult if study participants (or their proxies) had access to their raw data (7) and could verify that they were included in the study.

The possibility for research participants to access their raw data is a basic requirement for a just and reciprocal relationship, establishing at least a basic symmetry between those who donate and those who use data for their research (5, 6). Moreover, it enables individuals to act upon their considered judgments—a requirement for autonomous agency that was stated decades ago in the Belmont report (8). Providing access to the data that are derived directly from the sample, before analysis and interpretation, recognizes the donor's agency in at least three ways: freedom to decide, option of independent analysis, and informed decision about participation (see the box).

Besides these, there are many other good reasons for openness and reciprocal rela-

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ENACTING DONORS' AGENCY: THE BIOREPOSITORY AS AN EXAMPLE

Freedom to decide: Data donors can decide whether or not to access the raw data sets (e.g., the sequencing output data) from clinical and research biobanks. As no analysis has yet been performed, the role and responsibility of the biobank is merely to facilitate data access at a technical-procedural level. Usual disclaimers concerning data accuracy will apply, as technical errors can never be ruled out entirely.

Independent analysis: Data donors can, independently of the researchers and clinicians, apply data interpretation tools or have analyses performed. High-quality genome data–interpretation tools are available in the public domain, as well as commercial service providers. In particular, after analyses have been carried out and when filtered or selected findings are being disclosed, individuals may want to use the original raw data set for more integrated and comprehensive information or an independent opinion.

Informed decision about participation: The outcomes of an independent, participant-initiated analysis of the raw data can lead to better decisions about (continued) participation in a study or the pursuance of a diagnostic process. Later, the option of tracking who the data set has been shared with, and in what context, should be available. This, too, can contribute to a more informed decision about continued participation.

tionships between researchers and study participants (5, 6). Yet, on the basis of these three arguments alone, it is clear that providing access to their raw data is essential to taking individuals seriously as partners in research not merely as sources of samples and data. Giving individuals access to raw data is less complex than reporting research findings or returning the results of clinical investigations—issues that we do not claim to solve here.

A Crucial Distinction: Data Versus Findings

For a clearer perspective, we elaborate on the crucial distinction between access to data and returning of findings. The process of providing donors with access to their raw data (i.e., before any interpretation has been performed) is, in principle, straightforward. At the stage of returning findings, however, there are additional layers of complexity: At that point, people other than the data donors have (i) made the decision on how and which data are analyzed, (ii) on the relevance of findings, and (iii) whether they are actionable or not. In addition, at the data-processing stage, there are relevant differences between research and the clinic. These features have significant ethical and legal implications for the returning of results.

In research, notwithstanding the strict demand for data-sharing and transparency, researchers need to be able to protect data they are working on while discovery is in progress. This may temporarily override the interest of others in information-sharing and openness. Yet, these concerns do not apply to individual participants accessing their raw data.

In the clinical setting, there are further degrees of complexity, as the professional role, responsibility, and liability of clinicians and other actors must be accounted for. In

returning results of diagnostic procedures to patients, a health care provider assumes a specific professional responsibility that is clearly defined by the professional associations and is subject to legal regulations concerning the practice of medicine. There are concerns regarding the risks of individuals accessing data that they may misunderstand and impulsively act upon (9). However, long-term follow-up of direct-to-consumer genetic testing has not shown any evidence of such effects, and calls have been made to widen the notion of utility in this context to include personal or social utility (10, 11). Impulsive actions as a consequence of access to raw data are even less likely, because several intermediate steps are needed before substantial action is possible.

Another important question regarding current practice is whether anticipated difficulties in returning data justify the intentional limitation of knowledge generation and repeated cycles of information-filtering (3). Reduction of potential knowledge gain by avoiding or eliminating disturbing data points that may yield “unsolicited” (3) or “incidental” findings (1) is scientifically and clinically unsound and inhibits progress in the understanding of human biology and the complexity of disease states. Filtering out genetic variants that are assumed to have limited or no clinical utility, as recommended by the European Society of Human Genetics (3), systematically precludes the possible discovery of complex genetic causation.

Besides this, in the clinical setting, the lack of access to raw data directly derived from the donor's specimen is a relic of long-outdated strong paternalism in a practice of medicine where doctors were assumed to be omniscient. Giving a clear and focused answer to a specific question

from a patient—while at the same time communicating the uncertainty and incomplete knowledge inherent in medicine—is key in clinical practice. But dealing with the inevitable limits of knowledge in a constructive manner is fundamentally different from setting limits to data generation in the presumed “best interest” of the patient or as a way to mask uncertainty and limit the doctor's liability. The very terms used in discussions about when and how to best return findings to participants illustrate where the core agency lies: It lies with those who return and not with those who receive. Shifting the focus of the debate to access increases the scope of agency of research participants. The outcomes of that have shown to be without harm so far (5, 6, 10, 11).

Access to raw data is independent from the prospective delivery of interpreted information at a later point in time. Even the most thoughtful delivery of comprehensive information does not render obsolete a prior right (12) to access the raw data set.

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Acknowledgments: J.E.L. receives funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007–2013; REA grant no. 298698). The funding body and institutions had no role in the writing of the manuscript or in the decision to submit the manuscript for publication. The views expressed are entirely the authors' own. J.E.L. and G.M.C. thank the staff of the Personal Genome Project (PGP-Harvard) for discussion. All the authors thank J. Aach for helpful comments on an earlier version of this manuscript.

10.1126/science.1249382

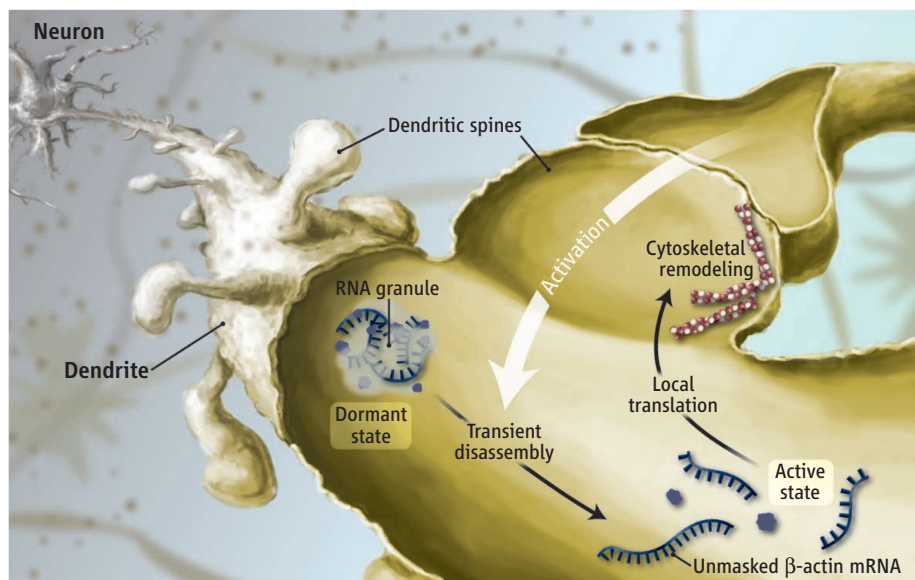
MOLECULAR BIOLOGY

mRNA, Live and Unmasked

Güney Akbalik and Erin M. Schuman

The localization of messenger RNA (mRNA) within a cell provides the opportunity for proteins to be expressed in specific subcellular compartments. This allows regions of the cell to be functionalized or modified in response to environmental cues. In neurons, long-term memory formation and synaptic plasticity require local protein synthesis at or near synapses (1). Granules comprising mRNAs and RNA binding proteins are transported within a cell, and their formation is regulated by signaling pathways (2, 3). On pages 422 and 419 in this issue, respectively, Park *et al.* (4) and Buxbaum *et al.* (5) visualize and characterize the dynamics of an endogenous mRNA. In neurons, mRNA encoding β -actin became transiently available for local translation after being released or unmasked from a latent complex. This first glimpse of endogenous mRNA behavior raises interesting questions about how RNA dynamics are coupled to translation.

In neuronal processes and the subcompartments of other cell types, mRNAs have been detected with a variety of techniques including biochemical analysis, in situ hybridization, and RNA sequencing (6). These approaches, however, do not provide direct information on the dynamic behavior of mRNAs. To observe mRNAs live, one must associate a fluorescent molecule (either a dye or a protein) to the mRNA of interest. For example, fluorescently labeled mRNAs that are injected into cells can be analyzed for their movement, including the speed of RNA granules as well as the molecular motors and the cytoskeletal elements they use to travel (7). Delivery of molecular beacons into cells is another possibility. This technique uses a small hairpin RNA probe labeled at its ends with a fluorescent dye and a quencher, yielding a signal only when the probe is bound to the mRNA of interest. However, this approach is not commonly used for live imaging because the signal is too low. Another approach is the expression of reporter proteins that are tagged with green fluorescent protein (GFP). Such fusion proteins can bind to RNA motifs located in the untranslated region (UTR) of an mRNA of interest (8). This method has the sensitiv-



Transient release. Synaptic activity in dendritic spines triggers the release of β -actin mRNA from granules and the localized synthesis of actin, enabling cytoskeletal remodeling during plasticity.

ity required to track single mRNA molecules. All of these techniques require the delivery of exogenous probes or reporters into cells, which tax cellular metabolism and often lead to cell toxicity and death (9).

To circumvent some of these problems, Park *et al.* genetically engineered a mouse in which the 3'UTR of endogenous mRNA that encodes the cytoskeletal protein β -actin was designed to contain 24 stem-loop structures. This mouse was crossed with a transgenic mouse expressing multiple copies of a GFP fusion protein that binds to the stem loops. The resulting progeny expressed β -actin mRNA molecules that were fluorescently labeled. This enabled Park *et al.* to monitor the movement of endogenous β -actin mRNA in living cells.

It is a relief to see that many of the measurements previously made for β -actin mRNA dynamics appear to be reasonable. The mean transport rate in fibroblasts was 1.3 $\mu\text{m/s}$, consistent with other findings (10). On the other hand, although the labeled endogenous β -actin mRNAs exhibited the same types of movements (stationary, diffusive, corralled, and directed), their relative proportions were different from earlier observations (10). Park *et al.* noted a reduction in the number of actively transported mRNAs in fibroblasts (22% versus 1%). This may result from differences in

Visualization of mRNA dynamics in live neurons reveals its release from granules at synapses during neuronal plasticity.

the reporters or cell types used. Discrepancy in the ratio of motion patterns was also seen between the fibroblasts and neurons.

What is the molecular composition of an RNA granule, and how is granule composition altered by neuronal activity? Some studies have shown that multiple exogenous mRNAs may assemble into a single granule (11). Other studies indicate that only a single mRNA is present in a granule (12). In neurons, Park *et al.* observed granules containing multiple β -actin mRNA copies in the soma and proximal dendrites, but a gradual decrease to a single copy in distant dendrites. By contrast, fibroblasts contained granules with a single copy of mRNA. In neurons, RNA granules exhibited two events during their movement: merge (joining into one granule) and split (separation from a parent granule). Membrane depolarization (which causes neuron activity) increased the ratio of split to merge events, consistent with the observed increase in granules containing single β -actin mRNA as well as the overall increase in granules.

Buxbaum *et al.* examined whether the above split-merge dynamics of RNA granules occurs during synaptic plasticity to liberate mRNAs for localized expression. High-resolution imaging of mRNA particles was combined with imaging of ribosomes

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and ribosomal RNA (rRNA) before and after a chemical induction of neuron plasticity. The authors observed a transient increase in mRNA and rRNA in dendrites upon neuron activation. This increase was mimicked by treatment with a protease and was not prevented by a transcription blocker, which suggests that the increase resulted from unmasking of RNA from RNA binding proteins, or disassembly of granules upon neuron activation, not from mRNA that was newly transcribed in the soma and then transported to dendrites. The unmasking event correlated with an increase in local β -actin synthesis; this suggests that the mRNA is in a latent protected state in the granule, which becomes unmasked for translation when the neuron undergoes plasticity. Such a mechanism may localize the expression of β -actin in dendrites and axons to promote cytoskeletal remodeling during synaptic plasticity or axon navigation.

To what extent do the properties described by Park *et al.* and Buxbaum *et al.* apply to all localized mRNAs? There has been much discussion as to how many mRNAs are actually packaged in granules (12). Park *et al.* and Buxbaum *et al.* make it clear that the number of mRNAs can also change over time and in space. What function do the other constituents of the mRNA-protein complex serve if the mRNA leaves the particle? Is the granule just a storehouse equipped for translational repression, or does it have other functions? The two studies suggest that there must be elements sensitive to signaling that allow the release of an mRNA from the granule. It was recently shown that mRNAs are repressed at the elongation step of translation in the granule, waiting to be reactivated for translation, contrary to the current assumption that they are repressed at the initiation step (13). Future studies should elucidate the number of times a single mRNA molecule can be translated.

Labeling techniques may also exert an influence on the speed, dynamics, and packing in an RNA granule. Anticipated improvements in labeling techniques, with brighter and smaller dyes, may refine our views.

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10.1126/science.1249623

CANCER

Hiding in Plain View—An Ancient Dog in the Modern World

Heidi G. Parker and Elaine A. Ostrander

A long-ago ancestor of the modern domestic dog is alive today in the form of the canine transmissible venereal tumor (CTVT). This tumor was first identified in the late 1800s when it was found to be transferred to new hosts through tumor cells (1). We now know that the tumor is naturally transmitted between dogs by direct contact, primarily through coitus or activities that permit sloughing of cells (2). The tumors are rarely metastatic and most tumors regress within a few months, leaving previously infected dogs with immunity. DNA analysis provides strong evidence that all CTVTs studied thus far are from a single source, one that existed before the dispersal of dog breeds around the world (3, 4). On page 437 of this issue, Murchison *et al.* (5) describe the first whole-genome sequence of CTVT, sampled from two tumors: one from a random-bred Australian Aboriginal camp dog and the other from a purebred American cocker spaniel from Brazil.

Sequence analysis of the two tumors revealed ~1.7 million somatic variants shared between them. These variants are presumed to have arisen during the initial malignant transformation or in the years of tumor passage before separation of the tumors by geographic boundaries. The number of somatic mutations is >100 times as large as the average mutation load of a human tumor, highlighting the long period over which mutations have accumulated and the number of alterations required to develop a stable colony of tumor cells. Mutations were scattered throughout the genome with more than 10,000 genes carrying at least one predicted protein-modifying genetic change. This list encompasses nearly half of the annotated genes from the dog reference sequence (6) and illuminates a cadre of genes and proteins necessary for cellular replication, maintenance of the tumor phenotype, and pathogenicity.

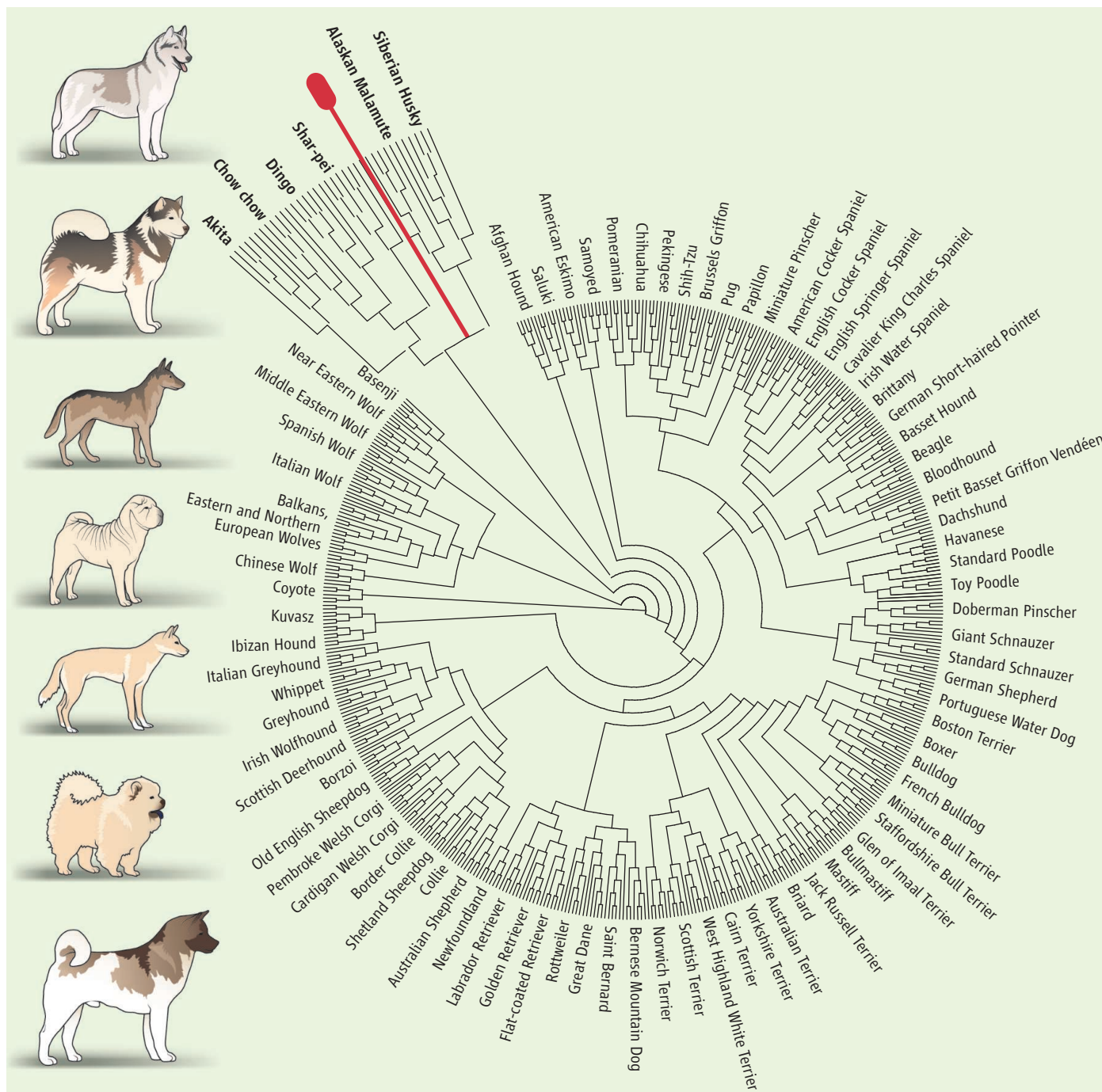
An examination of 23,782 single-nucleotide polymorphisms in both CTVTs and 1106 modern dogs and other canids places the origin of the tumor at ~11,000 years before present, with a second divergence of geographical tumor strains occurring <500

The sequence of a transmissible cancer provides a snapshot of a dog from the distant past.

years ago. Principal component analysis suggests that the first dog with CTVT was a member of the ancient dog group, the dog breeds closest to the wolf (7). Pairwise distance calculations place the Alaskan Malamute, a 4000-year-old breed that originally came to America from Eastern Asia, as the closest living relative of CTVT. In addition, the founder animal carried a mixture of “wolflike and doglike” alleles and was likely medium to large in size with short, straight fur, a black or agouti coat, prick ears, and a meso- to dolicocephalic head shape (see the figure). Dog fanciers will note that this description fits the modern Alaskan Malamute, but could also match that of any number of large spitz-type dogs.

Naturally occurring transmissible tumors are extremely rare. The only other known example is the Tasmanian devil facial tumor disease (DFTD), which was first identified in 1996 and has spread rapidly throughout the devil population. Unlike the canine tumor, DFTD is highly virulent, metastasizes readily, and is ultimately fatal (8). Sequencing of DFTD revealed a three- to eightfold increase in somatic mutations compared to human tumors as opposed to the >100-fold increase

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History of a transmissible tumor. Genomic analysis places the founder dog of CTVT within the ancient dog group. Dogs genetically most similar to the tumor (top to bottom): Siberian husky; Alaskan Malamute; depiction of the CTVT founder;

Shar-pei; dingo; Chow chow; and Akita. The neighbor-joining tree shows the placement of CTVT within a segment of the ancient dog group (expanded) with 80 modern dog breeds and wolves. The tree is modified from vonHoldt *et al.* (7).

in the canine tumor (9). This likely relates to the age of the tumor as DFTD has been acquiring mutations for ~20 years, whereas CTVT has been evolving for >10,000 years. In contrast to CTVT, DFTD is driving its population toward extinction, which will lead eventually to its own demise (8). It is possible that the canine tumor also started as an aggressive cancer, but that it evolved into a colonizing pathogen. A close comparison of

the mutations found in each tumor type might reveal variants important for understanding the unique behaviors of the two tumors.

One feature shared by both tumors is their appearance in populations of low diversity. The Tasmanian devil is a small island species with little genetic heterogeneity (10). Analysis of the major histocompatibility loci in CTVTs reveals that the source individual was inbred and largely homozygous

at the most variable dog leukocyte antigen haplotypes (3). The restricted gene pools in both founding populations may have aided the initial establishment of a clonally transmissible tumor. In the case of the dog, however, the population did not stay isolated, and the introduction of new major histocompatibility complex alleles may have kept tumor growth at bay. By comparison, Tasmanian devils are a small, closed popu-

lation, and there is therefore little hope of acquiring new resistance alleles.

For generations, the modern dog has served as little more than a culture medium for CTVT. The genome sequence of this living fossil will, together with the discovery of new mutations that control the phenotypes of modern dogs, provide deeper insight into the appearance and perhaps even the behavior of the ancient dog. In addition, the sequence analysis will reveal information about the evolutionary history of the tumor. For exam-

ple, CTVT has evolved methods of fooling the host's immune system, allowing it to establish a colony long enough for transfer between individuals to occur. Elucidation of these mechanisms based on the accumulation and position of mutations may help us understand the means by which pathogens escape host surveillance. This domestic dog tumor provides us with a unique system for answering some intriguing questions, making one wonder what else is hiding in the doghouse.

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10.1126/science.1248812

BIOCHEMISTRY

Making the H-Cluster from Scratch

Christopher J. Pickett

Many microorganisms use dihydrogen (H_2) as an energy vector between and within cells. This process is underpinned by FeFe and NiFe hydrogenases (1), which reversibly combine protons with electrons to give H_2 at very high rates. An understanding of the chemical mechanism by which they operate may lead to new technology for biological or bio-inspired catalysis (2, 3). Some 15 years ago, crystallographic and spectroscopic studies revealed the essential features of the active site of FeFe hydrogenase, the H-cluster (4). In the H-cluster, a dithiolate-bridged diiron subsite with cyanide and carbon monoxide ligands is linked through a cysteinyl bridge to a 4Fe4S cubane cluster (see the figure). On page 424 of this issue, Kuchenreuther *et*

al. (5) provide insights into the biosynthesis of this unusual structure.

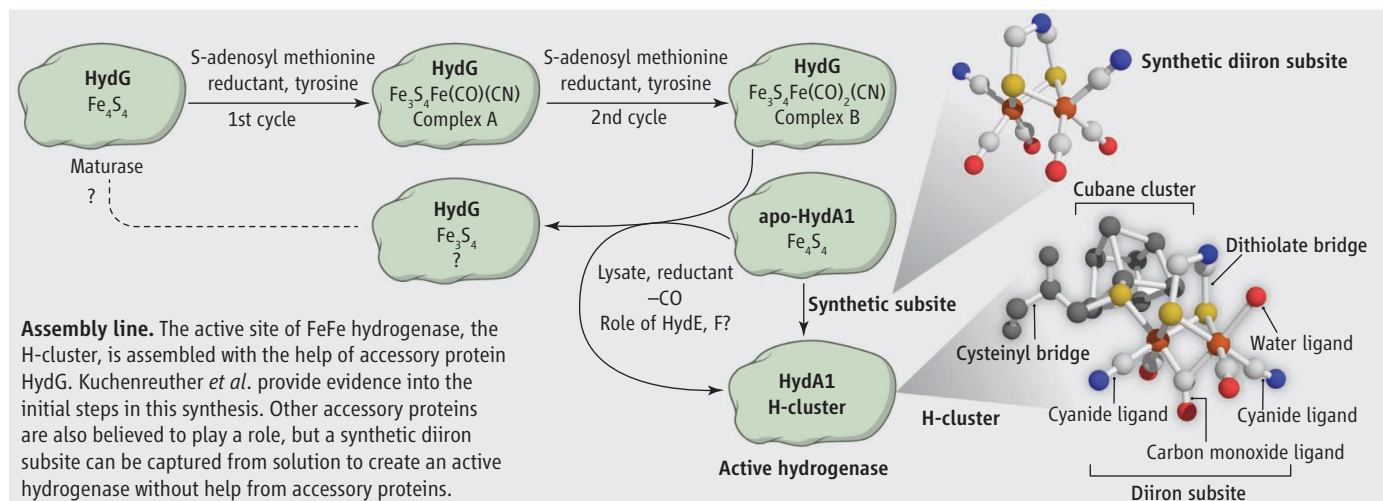
Extensive microbial, biochemical, chemical, and theoretical research is beginning to elucidate how FeFe hydrogenase works (1–4, 6), but it remains unclear how the H-cluster is assembled within the cell (7). In elegant Fourier-transform infrared and electron-nuclear double resonance spectroscopic work involving isotopic labeling, Kuchenreuther *et al.* show that the iron, carbon monoxide, and cyanide components of the subsite in active hydrogenase (HydA1) all originate from chemistry that occurs at a 4Fe4S cubane center of the accessory protein HydG. Transfer of these subsite components—with or without the aid of two other accessory proteins, HydE and HydF—transforms apo-HydA1 (which contains the cluster component of the H-cluster but not the subsite) to the functional hydrogenase HydA1.

Spectroscopic data begin to elucidate the initial steps in the biosynthesis of the hydrogenase active site.

HydG is a radical SAM (S-adenosyl methionine) enzyme that contains two 4Fe4S clusters. Formation of a 5'-deoxyadenosyl radical at one of the clusters leads to H-atom abstraction from tyrosine bound to the other cluster. The resulting dehydroglycine is cleaved to give the Fe(CO)(CN) unit of complex A. This unit is then converted to the Fe(CO)₂(CN) synthon of complex B. The latter is ultimately transferred to apo-HydA1 (see the figure). Kuchenreuther *et al.* suggest that the Fe(CO)(CN) and Fe(CO)₂(CN) moieties of complexes A and B are an integral part of the modified 4Fe4S framework of HydG as Fe₃S₄Fe(CO)_{1 or 2}(CN) clusters. This type of structure is unprecedented. However, chemical synthetic results (8) and observations on nitrogenase (9) lend support to the proposed structures.

The work of Kuchenreuther *et al.* extends the portfolio of organometallic radical SAM chemistry and opens up many exciting new

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questions. The formation of complex A requires one tyrosine in the first cycle of the enzyme. But once complex A is formed, the iron site at which this chemistry takes place is at least partially blocked by CO and CN and is electronically very different from that in the resting state of HydG. It remains to be shown how tyrosine is subsequently converted to CO to form complex B (together with a surplus CN⁻) in the second cycle of HydG, and how the Fe(CO)₂(CN) synthon is transferred and coupled to give the dithiolate-bridged subsite in the H-cluster.

By beginning to explain the early stages of H-cluster biosynthesis, the elegant spectroscopic study by Kuchenreuther *et al.* extends our knowledge of how the metallo-

sulfur active sites of a range of enzymes are assembled from simpler building blocks. The accessory proteins HydE, HydF, and HydG are all involved in the *in vivo* assembly of the active H-cluster on HydA (7) and must play a role in some or all of these steps. However, Kuchenreuther *et al.* have previously shown *in vitro* that HydG without HydE or HydF can activate an apo-hydrogenase in the presence of reductant and lysate (10). Given that in the native pathway, all the iron in the subsite moiety originates from HydG (5), it is plausible that a diiron subsite forms from two (or possibly one) HydG synthons, with subsequent release into solution and capture by apo-HydA1. Support for this idea comes from a study showing that a synthetic diiron

subsite (see the figure) (11) can be captured from solution by apo-HydA1 to give an active FeFe hydrogenase without participation of HydE or HydF (12).

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10.1126/science.1249276

ATMOSPHERIC SCIENCE

Climate Effects of Aerosol-Cloud Interactions

Daniel Rosenfeld^{1*}, Steven Sherwood², Robert Wood³, Leo Donner⁴

Aerosols counteract part of the warming effects of greenhouse gases, mostly by increasing the amount of sunlight reflected back to space. However, the ways in which aerosols affect climate through their interaction with clouds are complex and incompletely captured by climate models. As a result, the radiative forcing (that is, the perturbation to Earth's energy budget) caused by human activities is highly uncertain, making it difficult to predict the extent of global warming (1, 2). Recent advances have led to a more detailed understanding of aerosol-cloud interactions and their effects on climate, but further progress is hampered by limited observational capabilities and coarse-resolution climate models.

Recent advances have revealed a much more complicated picture of aerosol-cloud interactions (see the figure) than considered previously. For example, radiative forcing due to aerosol-cloud interactions may be limited by buffering mechanisms that result in compensation between different cloud responses to aerosols (3). Other situations may be hypersensitive to aerosols because

aerosols have become extremely depleted by precipitation (4). In these ultraclean regimes, addition of aerosols can dramatically increase cloud cover, causing a large cooling (5). Another newly appreciated process is aerosol-induced invigoration of deep convective clouds that may transport larger quantities of smaller ice particles to the anvils of such clouds. The higher, colder, and more expansive anvils can lead to warming by emitting less thermal radiation to space (6).

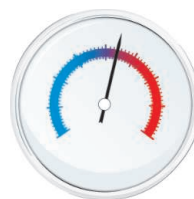
The Intergovernmental Panel on Climate Change's fifth assessment report (2) begins to account for some of these aerosol cloud-mediated effects. Most studies address a subset of known or suspected mechanisms, and they generally cannot separate individual contributions. Yet, this represents advancement with respect to the fourth assessment report (7), which accounted for only one specific effect: the aerosol-induced reduction of cloud drop size and the resultant increasing cloud solar reflectance. It is now clear that the reduced cloud drop size triggers other processes that may induce larger radiative perturbations than the droplet-size effect through mechanisms such as those depicted in the figure (8). The inability to fully quantify these effects increases the uncertainty in the radia-

Advances in satellite observations and model development are needed to disentangle the complex interactions of aerosols and clouds and their effects on climate.

tive forcing of aerosols and clouds. Furthermore, little is known about the unperturbed aerosol level that existed in the preindustrial era. This reference level is very important for estimating the radiative forcing from aerosols (9). Quantification of the reference level requires better quantitative understanding of the natural and anthropogenic emission sources and their interactions.

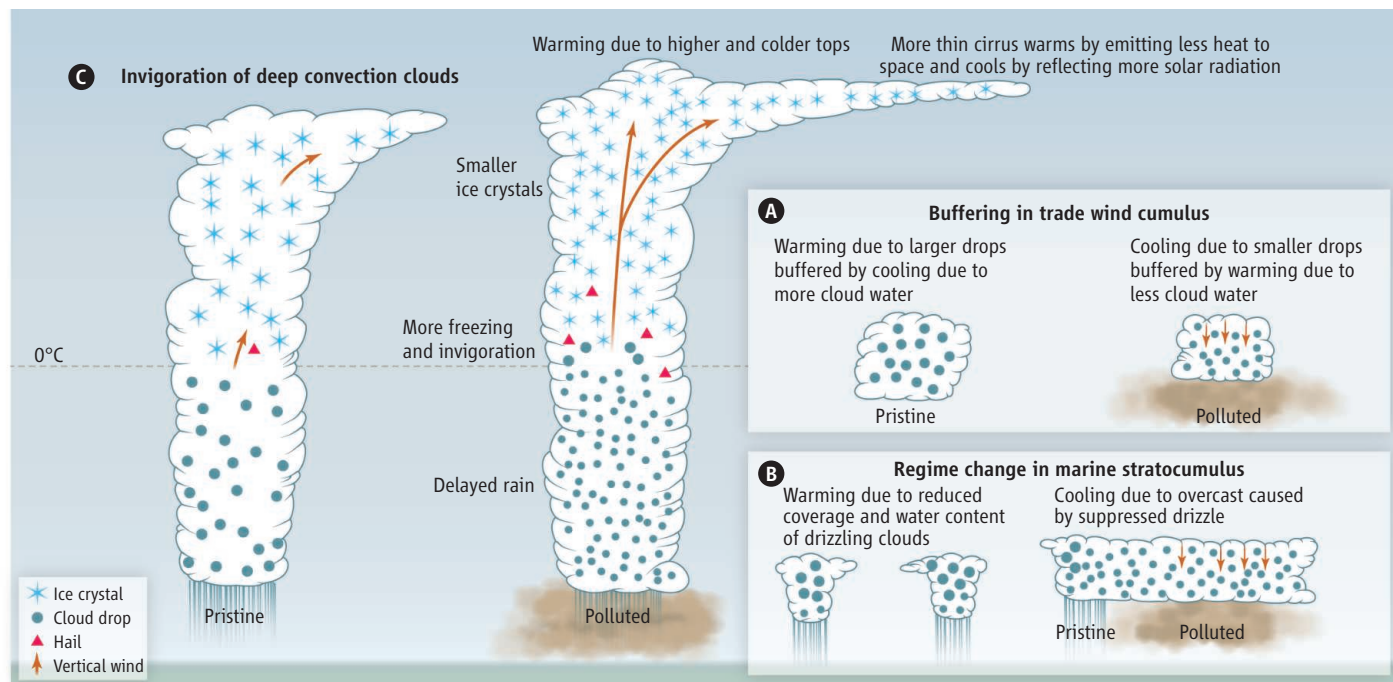
At fine scales (tens of meters or less), the processes by which aerosols alter the formation and growth of cloud drops and by which drops coalesce into rain are comparatively well understood, as are the ways in which turbulence affects these processes. Less clear is the response of the cloud cover and organization to the loss of water by rainfall. Understand-

ing of the formation of ice and its interactions with liquid droplets is even more limited, mainly due to poor ability to measure the ice-nucleating activity of aerosols and the subsequent ice-forming processes in clouds. Explicit computer simulations of these processes even at the scale of a whole cloud or multicloud system, let alone that of the planet, require hundreds of hours on the most powerful computers available. Modelers must therefore resort to simple parametric representations of these processes



Challenges in
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How aerosols affect the radiative properties of clouds. By nucleating a larger number of smaller cloud drops, aerosols affect cloud radiative forcing in various ways. **(A)** Buffering in nonprecipitating clouds. The smaller drops evaporate faster and cause more mixing of ambient air into the cloud top, which further enhances evaporation. **(B)** Strong cooling. Pristine cloud cover breaks up by losing water to rain that further cleanses the air in a positive feedback loop. Aerosols suppress-

ing precipitation prevent the breakup. **(C)** Larger and longer-lasting cirrus clouds. By delaying precipitation, aerosols can invigorate deep convective clouds and cause colder cloud tops that emit less thermal radiation. The smaller ice particles induced by the pollution aerosols precipitate more slowly from the anvils. This can cause larger and longer-lasting cirrus clouds, with opposite effects in the thermal and solar radiation. The net effect depends on the relative magnitudes.

in weather and climate models. Representing aerosol effects on clouds is particularly challenging, because small-scale correlated variations between aerosol and cloud properties have large-scale consequences, such as changes in cloud organization.

Fully resolved, global, multiyear simulations are not likely to become feasible for many decades. However, an exciting step was made in recent groundbreaking simulations (10), in which small domains capable of resolving cloud-scale processes, including simplified schemes of cloud aerosol interactions, were embedded in each grid cell of a climate model. This approach offers the potential for model runs that resolve clouds on a global scale for time scales up to several years, but climate simulations on a scale of a century are still not feasible. The embedded model is also too coarse to resolve many of the fundamental aerosol cloud processes at the scales on which they occur.

Improved observational tests are essential for validating the results of simulations and ensuring that modeling developments are on the right track. Current satellites can measure cloud and precipitation properties but not the vertical winds that create the clouds, nor the specific aerosols on which the cloud drops and ice crystals nucleate. Therefore, it is difficult to disentangle the aerosol and

meteorological effects on cloud properties. A major challenge is that the most important aerosol nucleation region is at the bottom of a cloud, which is obscured by the rest of the cloud if measured from above.

The Cloud-Aerosol Lidar and Infra-red Pathfinder Satellite Observation (CALIPSO) (11) and EarthCare satellite missions aim to address some of these challenges. EarthCare (estimated to launch in 2015) will measure vertical profiles of aerosol types and amounts with a 355-nm lidar. EarthCare's Doppler cloud radar can determine cloud vertical motions. However, the radar and lidar in both missions cover only the line of subsatellite track, limiting their coverage. Further satellite missions that overcome the measurement challenges are being considered (12).

Progress on understanding aerosol-cloud interactions and their effects on climate is limited by inadequate observational tools and models (13). Yet, achieving the required improvement in observations and simulations is within our technological reach. For example, available technology could provide multispectral and multiangle polarimetric measurements of cloud properties at a resolution of 100 m (12). The level of effort should match the socioeconomic importance of what the results could pro-

vide: lower uncertainty in anthropogenic climate forcing and better understanding and predictions of future impacts of aerosols on our weather and climate.

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Acknowledgments: D.R. is partially supported by project BACCHUS FP7-603445. The authors appreciate V. Ramaswamy's comments on this Perspective.

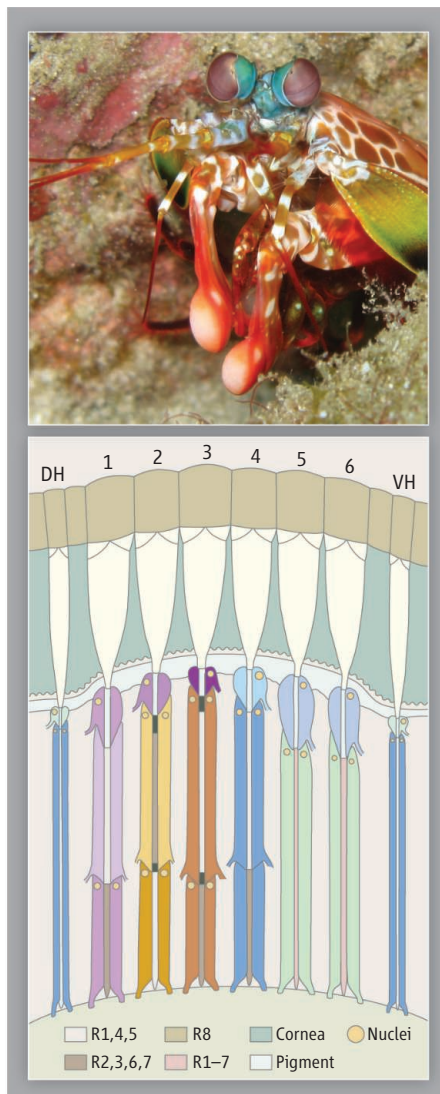
PHYSIOLOGY

Extraordinary Color Vision

Michael F. Land and Daniel Osorio

How do animals see color? We may never know how another animal experiences red or blue, but we do know that sensitivity to ultraviolet light allows bees to see patterns on flowers where we see plain yellow or white. In fact, bee and human color vision are much alike. Both have three spectral types of photoreceptor whose signals are compared by neural “opponent” mechanisms, which are sensitive to the relative amounts of light at different wavelengths, allowing the animal to distinguish the spectrum of a light source from its brightness. Thomas Young (1) recognized in 1802 that having multiple receptors each with a different spectral sensitivity at each point in the image is essential for color vision, but inevitably impairs spatial resolution. He proposed three spectral receptors as the likely compromise. In fact, theory predicts that two to four receptor types are optimal for discriminating the spectra of natural materials and maximizing the number of objects that could be distinguished by color (2). The eyes of many animals seem to follow these principles, with a retina containing two to four spectral receptors combined with a neural mechanism to compare the responses of different receptor types (the color opponent process). It is therefore fascinating to find an animal that sees color in a fundamentally different way, as reported by Thoen *et al.* on page 411 of this issue (3).

Stomatopods are an ancient group of crustaceans that mainly live in tropical coral reefs. They are known as mantis shrimps because of a pair of claws that, like those of a praying mantis, unfold with great speed to capture prey (4). This behavior is directed by a pair of eyes that are unique in the animal kingdom. For the most part these eyes are of an apposition construction, common to most diurnal crustaceans and insects, where each facet-lens contributes to a single point (or pixel) in the visual image. However, running through the center of each eye is a “midband” of six ommatidial rows—which in most reef-living stomatopods contain four rows that make up a color vision system with 12 visual pigments—as well as two rows that analyze both linearly and circularly polarized



A new way of seeing in color. The mantis shrimp *Odontodactylus scyllarus* and a diagram of a section through six rows of the compound eye midband. The colors indicate the approximate spectral sensitivities of the photoreceptors (3, 5). DH, dorsal hemisphere; VH, ventral hemisphere.

light (see the figure) (5, 6). The ommatidia that deal with color have a three-tier structure: The top tier contains an ultraviolet-sensitive pigment, and below this are two further tiers containing different pigments sensitive to wavelengths in (our) visible spectrum (see the figure). The two central rows have, in addition, two sets of color filters that sharpen the spectral sensitivities of the underlying receptor pigments. This gives the

The compound eyes of mantis shrimps see color in a fundamentally different way from other animals.

mantis shrimps a color vision system with 12 spectral channels. The upper and lower halves of each eye also have overlapping visual fields, so that each eye can act as a stereoscopic rangefinder, at least for close distances (7).

The field of view of the midband is 180° long but only about 5° wide; it provides a system that can analyze the light spectrum reaching it in detail, but only along one line in space. Thus, whereas most eyes encode color and form in the same part of the retina, for the mantis shrimp to determine the color or polarization of an object its eye muscles must scan the band across the scene. Indeed, stomatopods have small, slow scanning movements that allow the midband to scrutinize a particular area (8).

Stomatopods live in a colorful world and probably use their colorful appendages as recognition badges (9). The uropods at the rear also have interesting polarizing properties. Thus, the extraordinary sensory machinery of the eye midband may be useful for species, and even individual, recognition, as well as more generally in detecting and recognizing prey and other objects.

Thoen *et al.* examined color vision in the stomatopod *Haptosquilla trispinosa*. When *Haptosquilla* is trained to associate a spectral light with a food reward, it can discriminate wavelength differences of about 25 nm, which corresponds roughly to the difference between what we see as pure yellow and orange. By comparison, humans can discriminate wavelength differences of 1 to 4 nm across the spectrum, with two distinct minima (10). This spectral sensitivity can be attributed to the tuning of the red, green, and blue receptors followed by color-opponent mechanisms for comparing their outputs (11). Thus, despite having 12 narrowly tuned spectral receptors, *Haptosquilla*'s wavelength discrimination is poor, and Thoen *et al.* find no evidence for opponent interactions in the wavelength discrimination function. To account for these findings, they suggest that mantis shrimps' remarkable eye avoids the need for complex neural processing, instead identifying objects by using the signals from single midband receptor types as they are scanned over a scene.

It is appealing to suggest that sense organs should save on neural processing,

which may be metabolically costly or evolutionarily complex. Even so, it is not clear how the signals in the 12 midband spectra receptors are read out by the brain. Natural spectra are not monochromatic and the animal would need to distinguish changes in brightness, perhaps caused by high-lights and shadows, from colorful objects. The visual system would have to compare the signals in single midband receptors with those of others, as in normal color opponency. A simple way would be divide the response of each receptor type by an overall brightness signal derived by summing the outputs of all midband receptors.

An alternative is to compare signals of the three receptor types in each midband row, in effect giving the animal four independent trichromatic systems.

The poor color discrimination found (3) is also a puzzle. It would be interesting to understand how stomatopods use color in their daily lives. For instance, in conflicts mantis shrimps seem to assess their opponent's color to decide whether to fight, allowing them to avoid dangerous opponents (9). If so, they are likely to need to discriminate smaller differences than those found when they are trained to associate color with food (3).

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10.1126/science.1249614

PHYSICS

Lifting the Fog of Complexity

Dirk K. Morr

Identifying the microscopic origin of materials' complex properties is one of the main intellectual challenges in condensed matter physics. The emergence of complexity is often tied to novel forms of collective behavior driven by strong interactions. Unconventional superconductors, such as the high-temperature (cuprate) or iron pnictide superconductors, and heavy fermion compounds are archetypical examples for materials in which the competition and entwining of collective forms of behavior give rise not only to a complex phase diagram but also to unexpected properties that have resisted all attempts of a theoretical explanation. In the phase diagram of the

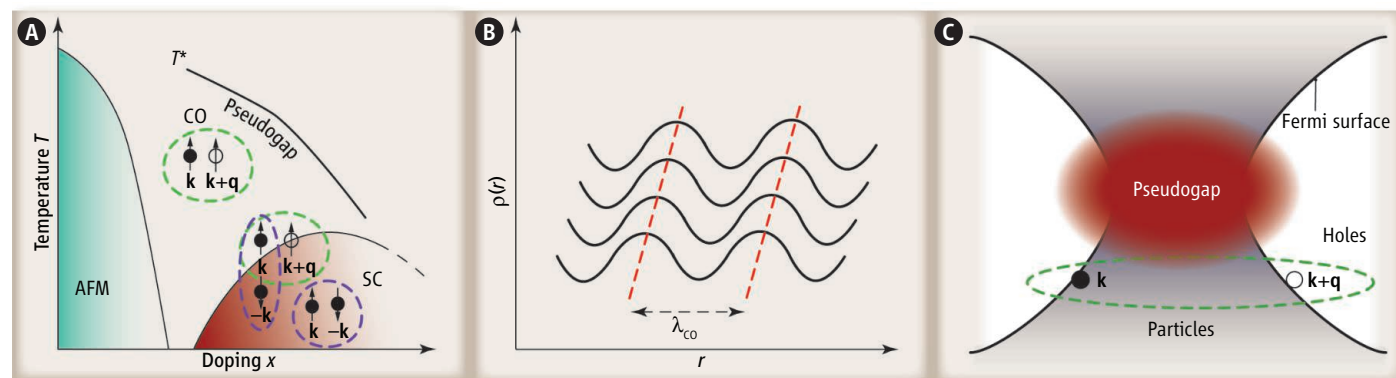
cuprate superconductors, these properties are associated with the pseudogap region, a region located in close proximity to magnetic, charge, and superconducting orders. Whether the competing nature of these various orders is the underlying cause for the pseudogap and its unconventional properties is one of the central questions in this field. On pages 390 and 393 in this issue, Comin *et al.* (1) and da Silva Neto *et al.* (2) answer a crucial part of this question by elucidating the relation among superconductivity, charge order, and the pseudogap region, as well as establishing the ubiquitous nature of charge order across different families of cuprate superconductors.

The cuprates can be tuned from insulating antiferromagnets to unconventional superconductors via doping (chemical sub-

Combining surface- and bulk-sensitive experimental probes may help to solve the complexity underlying high-temperature superconductivity.

stitution of elements), giving rise to a complex phase diagram (see the figure, panel A). Between the antiferromagnetic (AFM) and superconducting (SC) phases resides the pseudogap region, where many of the material's properties strongly deviate from those predicted by Landau-Fermi liquid theory, one of the cornerstones of condensed matter physics (3). The region's name arises from the (incomplete) transfer of the material's quantum states from low to high energies, which creates an energetic pseudogap. The proximity of the pseudogap region to antiferromagnetism and superconductivity, combined with the observation of static charge order (CO) in some cuprate families (4), has given rise to the suggestion that the pseudogap region represents an incipient charge (5), magnetic (6), or superconduct-

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Unraveling complexity. (A) Schematic phase diagram of the cuprate superconductors. Charge order (CO) involves pairing of an electron (solid circle) and hole (empty circle) with the same spin $\uparrow$ and momenta $\mathbf{k}$ and $\mathbf{k} + \mathbf{q}$ (green dashed ellipse), whereas superconductivity arises from pairing of two electrons with opposite spins,

$\uparrow$ and $\downarrow$, and momenta $\mathbf{k}$ and $-\mathbf{k}$ (blue dashed ellipse). (B) Charge order implies spatial oscillations in the electron density, $p(r)$. (C) Quantum states (in momentum space) involved in the formation of charge order (electron, solid black circle; hole, open black circle) and the quantum states affected by the pseudogap (red area).

ing order (7), arises from competing orders (8), or is tied to an insulating Mott state (9).

Trying to identify the relation among charge order, magnetic order, superconducting order, and the pseudogap region presents a “chicken-or-egg” problem: Does the proximity to magnetic order induce superconductivity and the pseudogap, or does the pseudogap facilitate charge order, thereby suppressing both magnetism and superconductivity? Although these questions have been the topic of many debates, no consensus has been reached to date. Lifting this “fog of complexity” is important, as it is common to many strongly interacting systems in condensed matter physics, nuclear physics (10), and biology (11).

The experiments by Comin *et al.* and da Silva Neto *et al.* provide a powerful new approach to address this issue. Combining surface-sensitive scanning tunneling microscopy (STM) and angle-resolved photoemission spectroscopy (ARPES) experiments with bulk resonant elastic x-ray scattering (REXS) has enabled a comprehensive view of the relation among charge order, superconductivity, and the pseudogap in the bismuth-based family of high-temperature superconductors. With STM experiments probing the spatial structure of charge oscillations (see the figure, panel B) and REXS allowing the complementary detection of these oscillations in momentum space, the emergence of a dynamical charge order below a characteristic temperature T_{CO} is demonstrated. The spatial wavelength λ_{CO} of these charge oscillations is similar to that observed in La-based (4) and Y-based (12) cuprate superconductors; hence, this work establishes that dynamical charge order is a ubiquitous phenomenon in the high-temperature superconductors.

The observation by Comin *et al.* that T_{CO} approximately coincides with the onset temperature T^* of the pseudogap implies a close relationship between charge order and the pseudogap. However, which of them is the chicken and which is the egg? To answer this question, both groups investigated the quantum states taking part in the formation of these two phenomena. Charge order emerges from the pairing of an electron of momentum $\mathbf{k}$ with a hole (a missing electron) of momentum $\mathbf{k} + \mathbf{q}$, where $|\mathbf{q}| = 2\pi/\lambda_{CO}$ (see the figure, panel A). The electron and hole involved in the pairing process are located near the Fermi surface, an imaginary line in momentum space separating electrons and holes. By using the Fermi surface extracted from ARPES experiments, both groups identified the electron

and hole quantum states participating in the formation of the dynamical charge order (see the figure, panel C). Are these quantum states the same as those being affected by the emergence of the pseudogap? Both groups provide a resounding no to this question: The quantum states involved in the formation of the charge order lie outside the region in momentum space that is involved in the formation of the pseudogap (see the figure, panel C). The conclusion is that it is the pseudogap that facilitates the formation of the charge order.

Another important part of the puzzle is revealed by da Silva Neto *et al.* through the observation that with the onset of superconductivity at T_c , the strength of the charge order weakens, ultimately vanishing at temperatures well below T_c ; this demonstrates the competing nature of superconductivity and charge order (12). Because superconductivity requires the pairing of an electron with another electron of opposite spin and momentum, the results of da Silva Neto *et al.* imply that the pairing partner of an electron changes from being a hole at temperatures above T_c (giving rise to charge order) to being an electron below T_c (giving rise to superconductivity), with some superposition of these two types of pairing possible in the vicinity of T_c (see the figure, panel A). The competition between charge order and superconducting order therefore reflects the

nature of two competing pairing tendencies that cannot be simultaneously satisfied.

The studies by Comin *et al.* and da Silva Neto *et al.* represent a major breakthrough in dissolving the fog of complexity surrounding the cuprate superconductors. Nonetheless, many questions still remain: What is the origin of the pseudogap? Is the competition between different types of order responsible for the cuprates' many unconventional properties? The comprehensive, multipronged approach adopted by Comin *et al.* and da Silva Neto *et al.* is a promising way forward in investigating these and many other open questions posed by complex systems.

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10.1126/science.1248868

IMMUNOLOGY

The Fiery Side of HIV-Induced T Cell Death

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A cytosolic protein that senses fragments of HIV-1 DNA triggers the death of uninfected CD4 T cells.

In untreated HIV-1 infection, the progressive destruction of CD4 T cells gives rise to a devastating number of opportunistic infections—the hallmark of AIDS. Yet, it remains unclear how HIV-1 inexorably depletes these essential immune cells. Most of the dying CD4 T cells are uninfected “bystanders” that self-destruct upon exposure to HIV-1 DNA products generated during aborted infection (1). Doitsh *et al.* (2) and

a report by Monroe *et al.* on page 428 in this issue (3) show that these truncated fragments of HIV-1 DNA trigger CD4 T cell demise through an intense inflammatory cell death pathway—called pyroptosis—after their recognition by an intracellular DNA sensor (4, 5).

Although HIV-1–induced CD4 T cell death had long been attributed to apoptosis (6, 7), two host cell proteases, caspase-1 and caspase-3, are activated in bystander CD4 T cells by nascent HIV-1 genomic DNA (1). Whereas caspase-3 is associated with the classical pathway of apoptotic cell death, caspase-1 elicits “pyroptosis” (from the Greek for “fire fall-

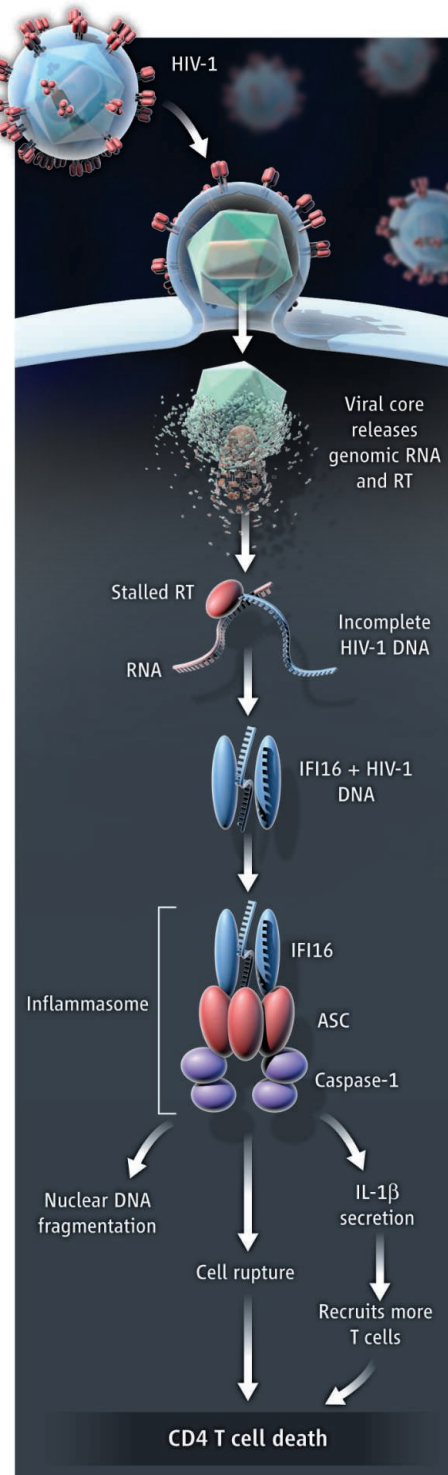
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ing). Caspase-1 is activated by a multiprotein complex, the inflammasome, which orchestrates antipathogenic responses (5, 8–10). However, it was not clear whether one or both of these death pathways delivered the fatal blow to bystander CD4 T cells. Doitsh *et al.* (2) now show that the mode of cell death is determined by how permissive the CD4 T cells are to HIV-1 infection. A small proportion (<5%) of activated CD4 T cells that are productively infected die by caspase-3–dependent apoptosis, but the vast majority of nonpermissive, quiescent cells perish as a result of caspase-1–elicited pyroptosis. The HIV-1 resistance of bystander CD4 T cells arises in part from a host cell deoxynucleoside triphosphate (dNTP) triphosphohydrolase [called sterile alpha motif (SAM) domain and histidine–aspartate (HD) domain–containing protein 1 (SAMHD1)]. This enzyme depletes cytosolic dNTP pools, thus depriving HIV-1 of essential building blocks (11). Ironically, the incomplete viral DNA genomes produced as a consequence of SAMHD1’s protective inhibition of HIV-1 now appear to initiate pyroptosis as well as the elaboration of the inflammatory cytokine interleukin-1 β (IL-1 β), which lures even more CD4 T cells into the killing zone (1, 2).

What host cell protein senses these incomplete strands of HIV-1 DNA and throws on the self-destruct switch? Monroe *et al.* used DNA affinity chromatography to identify six potential candidates. Previous studies had characterized these factors as DNA binding proteins. Among these was interferon- γ -inducible protein 16 (IFI16), which quickly moved to the top of the suspect list given its known role in forming an inflammasome in response to herpesviruses and intracellular DNA (12, 13). When the authors inhibited IFI16 expression using RNA interference, bystander CD4 T cell death was avoided to an extent similar to that achieved by drugs that block HIV-1 DNA production. The IFI16-depleted CD4 T cells also displayed lower amounts of active caspase-1 and inflammatory cytokines, consistent with decreased pyroptosis, and demonstrating that the relevant sensor was in hand.

Monroe *et al.* further elucidated that IFI16 played a nonredundant role in mediating bystander CD4 T cell death by ruling out three other sensor candidates that had also bound to HIV-1 DNA. Collectively, these findings highlight the potentially critical roles of IFI16, the inflammasome, and pyroptosis in the progression of HIV-1 infection to AIDS.

The findings of Doitsh *et al.* and Monroe *et al.* also underscore the double-edged sword of immune defenses. IFI16 recog-



nizes the DNA of multiple herpesviruses, leading to the curtailment of their replication (12, 13). However, when similarly activated by the DNA of HIV-1, this same sensor sets in motion the depletion of a crucial class of relatively resistant CD4 T cells, thereby substantially contributing to the AIDS pandemic. The flipside of this scenario is that individuals with inactivating polymorphisms in IFI16, though more vulnerable to herpesviruses, may nonetheless

T cell suicide. Nonproductive HIV-1 infection of quiescent “bystander” CD4 T cells generates incomplete HIV-1 DNA genomes that are recognized by the host DNA sensor IFI16. IFI16 then forms an inflammasome with apoptosis-associated speck-like protein (ASC), resulting in the activation of caspase-1. In turn, activated caspase-1 elicits pyroptosis, which includes nuclear DNA fragmentation, cytokine production (IL-1 β), and cell rupture. The consequences are cell death and an inflammatory cycle that attracts and destroys additional CD4 T cells, leading to disease progression. Although the host factor SAMHD1 depletes dNTPs and thereby inhibits the viral reverse transcriptase (RT) (not shown), there are enough abbreviated HIV-1 genomes to elicit cellular suicide.

be protected from CD4 T cell loss during HIV-1 infection.

Indeed, the studies by Doitsh *et al.* and Monroe *et al.* may set the stage for a new class of anti-HIV-1 therapeutics, as demonstrated by the caspase-1 inhibitor VX-765, which prevents CD4 T cell pyroptosis and death (2, 14). The identification of this effective and clinically safe inhibitor provides exciting opportunities to perform in vivo studies that explore the contribution of pyroptosis to CD4 T cell decline. Future efforts will likely address whether pyroptosis inhibition has a clinical indication, together with existing antiretroviral therapy, in preventing disease progression. Such a prospect would be a welcome addition to the armamentarium of current therapeutics by uniquely targeting the self-destructive consequences of this damaging inflammatory response. In addition, by acting upon a host target, these anti-inflammatory therapies would circumvent viral resistance, thereby providing a potentially durable approach to prevent AIDS.

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Acknowledgments: We thank C.M. Sasseti and K.A. Fitzgerald for helpful discussions.

10.1126/science.1250175

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A Paleogenomic Perspective on Evolution and Gene Function: New Insights from Ancient DNA

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The publication of partial and complete paleogenomes within the last few years has reinvigorated research in ancient DNA. No longer limited to short fragments of mitochondrial DNA, inference of evolutionary processes through time can now be investigated from genome-wide data sampled as far back as 700,000 years. Tremendous insights have been made, in particular regarding the hominin lineage. With rare exception, however, a paleogenomic perspective has been mired by the quality and quantity of recoverable DNA. Though conceptually simple, extracting ancient DNA remains challenging, and sequencing ancient genomes to high coverage remains prohibitively expensive for most laboratories. Still, with improvements in DNA isolation and declining sequencing costs, the taxonomic and geographic purview of paleogenomics is expanding at a rapid pace. With improved capacity to screen large numbers of samples for those with high proportions of endogenous ancient DNA, paleogenomics is poised to become a key technology to better understand recent evolutionary events.

The field of molecular genetics that studies ancient DNA has been among those most dramatically transformed by high-throughput, “next-generation” DNA sequencing (NGS) technologies (Fig. 1). Within the last several years, these technologies have made it possible to sequence and assemble ancient genomes (Table 1), an accomplishment that for much of the history of the field was widely believed to be impossible.

The first ancient DNA sequences were reported three decades ago from a museum-preserved skin of the extinct quagga (1), and, nearly simultaneously, from an Egyptian mummy (2). Although the latter is now widely accepted to be the result of contamination, highlighting a major issue to be overcome, these early studies garnered enthusiasm to obtain DNA from fossils. The invention of the polymerase chain reaction (PCR), which amplifies nucleic acids (3), soon made it possible to target specific sequences, allowing for replication and validation. Not surprisingly, many of the most improbable results—DNA from dinosaurs and amber, for example—could not be validated and are now known to have been the result of contamination (4, 5). In response, the ancient DNA community adopted a suite of criteria for authenticating data (6, 7). These included replicability—if an ancient DNA sequence is real, it should be possible to reproduce it—and reliability—replicates of the same target sequence should be identical. Although some early ancient DNA studies targeted nuclear DNA (8–10), an-

cient nuclear DNA sequences authenticated with these criteria were rare.

The DNA sequences discussed here are labeled “ancient,” but this classification is less about age than about biochemical condition. Most recoverable fragments of ancient DNA are shorter than 100 base pairs (bp) in length (11) and contain miscoding lesions (12–14) that can result in erroneous sequences. Although it was predicted that ancient DNA would not survive for more than 100,000 years (15), it is now known that DNA can survive nearly an order of magnitude longer than that (16–18).

Ancient DNA makes it possible to observe changes in genetic diversity through time. It can be used to test hypotheses about the relationships between environmental events and evolutionary changes in populations [e.g., (19–21)]. It can also resolve controversy about evolutionary relationships between species [e.g., (22–25)] and provide calibrations for the molecular clock [e.g., (26)]. However, as ancient DNA has remained restricted primarily to high-copy number mitochondrial and chloroplast DNA, these inferences tend to come from single loci. Without access to the nuclear genome, it is not possible to infer extinct phenotypes, detect episodes of selection, or investigate hypotheses about ancient admixture.

With a complete genome, however, it is possible to infer even complex evolutionary relationships (Fig. 2). For example, if their age is known, paleogenomes can resolve and provide calibration for molecular phylogenies, as in a recent study of horse evolution (Fig. 2A) (18). If sequenced to sufficiently high coverage, paleogenomes can be used to infer long-term demographic trends. For example, using coalescent theory combined with genome-wide heterozygosity (27), the demographic history of the Denisovans, an archaic hominin group known only from Denisova cave in the Altai Mountains in Siberia,

was inferred (Fig. 2B) (28). Paleogenomic data can also be used to reveal otherwise cryptic relationships between past and present populations. Most notable has been the discovery of admixture between Neandertals, Denisovans, and anatomically modern humans (29–31). As greater numbers of paleogenomes become available, it is likely that similar situations will be revealed for other taxa, providing increased power to understand the relationship between environmental change and biodiversity (Fig. 2C). Beyond demographic inferences, paleogenomes can be used to identify selection within the genome, including genetic changes that may underlie species-specific traits (Fig. 2D). For example, 367 mutations in genes, regulatory regions, and splice sites that have become fixed in humans since divergence from Denisovans were identified from the Denisova genome (28), presenting potential targets for future functional analyses. Finally, paleogenomes provide a means to investigate genome evolution (32), including the evolution of pathogenicity (33–38).

The First Paleogenomes and the Enduring Curse of Contamination

In 2005, a high-throughput approach was used to sequence ~15,000 bacterial colonies containing DNA sampled from two ~40,000-year-old Austrian cave bears (39). The result was a mixed sample of cave bear, bacteria, fungi, plant, and other sequences, where less than 6% of the recovered DNA was determined to be that of cave bear (39). Nevertheless, the 27 kb of cave bear nuclear DNA established that, in principle, it would be possible to sequence and assemble a paleogenome.

The low percentage of endogenous DNA in these samples is not surprising. When an organism dies, its DNA begins to decay almost immediately and continues to decay at a rate determined by the environment (15, 40). Cold, dry environments discourage the growth of microorganisms and minimize chemical damage. Remains that are quickly buried and, ideally, frozen tend to be best preserved. Extracted ancient DNA is always a mixture of organismal and environmental DNA, including DNA from bacteria, fungi, and other organisms that colonize the sample during burial, and any contamination occurring during excavation and processing. However, low endogenous percentages do not rule out paleogenomic analysis. For example, paleogenomic data were used to infer the evolutionary relationship between a 6000-year-old *Myotragus* (an extinct bovid) and other bovid species despite 0.27% endogenous content (41). Even lower values (0.01 to 0.03% endogenous DNA) were reported from a ~40,000-year-old human bone from Tianyuan Cave, China, and yet a complete mitochondrial genome and several nuclear loci were reconstructed (42). Although samples with more endogenous DNA are better targets for sequencing, endogenous DNA content varies widely, even between samples with similar preservation histories (43–45), making sample selection a difficult but important step.

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The first paleogenomic studies using NGS produced ~13 Mb of nuclear DNA from a 28,000-year-old mammoth fossil (46) and ~1 Mb of Neandertal DNA (47). A simultaneous project using bacterial cloning to sequence the same Neandertal extract produced ~60 kb of Neandertal nuclear DNA. Among the two Neandertal studies, the study that used bacterial cloning inferred an older common ancestor for the lineages leading to humans and Neandertals. A reanalysis of the NGS-derived data (48) suggested that more than 50% of the sequences may have been contaminants from modern humans.

Remains can become contaminated with human DNA at any point during excavation, storage, and processing. The most reliable methods to estimate contamination do so directly, by identifying sequence motifs that differ between the paleogenome and the potential contaminant and then calculating the proportion of contaminating sequences (49). This approach was used to estimate the amount of contamination in the mitochondrial component of the NGS Neandertal data set (47). Positions that differed between the newly available, complete Neandertal mitochondrial

genome and humans were identified and counted, and it was estimated that ~11% of the original mitochondrial data were modern human contaminants (50). This direct approach is now widely used in paleogenomic analyses [e.g., (29, 31, 51)].

Although the source of contamination in the first NGS-derived Neandertal data set remains unknown and later Neandertal research has superseded these early data, the issue provided an important lesson to the paleogenomics community: The sequencing library was not prepared in a sterile laboratory (50), and this may have provided an opportunity for contamination. Consequently, paleogenomic libraries are now routinely prepared in dedicated ancient DNA facilities.

Hominin Paleogenomics

In 2010, a 20-fold coverage genome of a 4000-year-old paleo-Eskimo from Greenland’s Saqqaq culture was isolated from a tuft of hair (51). Hair is a good source of ancient DNA because its hydrophobic exterior limits colonization by bacteria and makes it possible to clean the surface before extraction (52). In assembling these data, a potential general limitation of paleogenomics was re-

vealed: Even with 20-fold coverage, only 79% of the Saqqaq genome could be determined. This is likely a consequence of the short length of ancient DNA fragments (an average for the Saqqaq specimen of 55 bp) (51). Although there is no strict rule, most very short fragments cannot be mapped unambiguously to a single location in a genome, particularly when that genome is highly repetitive, as are most eukaryotic genomes. Unfortunately, most ancient sequences are as short as or shorter than the Saqqaq sequences [e.g., (17, 28, 29)] (Table 1). Even those isolated from a tuft of 100-year-old hair from an Australian aborigine were, on average, only 69 bp long, despite the specimen’s young age (53). Given the challenge of accurately mapping short reads to a reference genome, it has been standard in paleogenomic assemblies to discard sequence fragments <30 bp in length (17, 28, 29). This suggests that, even with improved methodologies to recover the shortest surviving DNA fragments (17), it may not be possible to sequence any eukaryotic paleogenome truly to completion.

Soon after the Saqqaq paleogenome, a 1.3-fold coverage Neandertal genome (29) was produced

	Pre-NGS	NGS	Present	Future
Genomic data generated	• Single-locus data sets • Multicopy loci	• Low-coverage paleogenomes Complete genome	• High-coverage paleogenomes Proportion of genome recovered	• Multiple, high-coverage paleogenomes per species • Increased taxonomic and temporal diversity of paleogenomes
Post-processing	• Screening for contaminants and damage by eye	• Bioinformatic awareness of damage and ancient DNA-specific substitution profiles	• Improved pipelines for reference-guided and de novo assemblies	• Better characterization of DNA damage (single-molecule) • Detection of methylated sites • Greater taxonomic diversity of scaffold genomes
Sequencing	• Sanger sequencing • Molecular cloning	• High-throughput amplicon sequencing • Shotgun sequencing	• Increased throughput • Lower per-base cost	• Increased throughput • Lower per-base cost • Single-molecule sequencing
Pre-processing	• PCR	• Library preparation • Targeted enrichment	• Single-stranded library preparation	• Increased efficiency • Blocking strategies for non-endogenous DNA and repeats
Nucleic acid extraction	• Silica-based • Phenol:chloroform DNA recovered	• Optimized silica-based protocol	• Increased recovery of very short fragments	• Increased efficiency • DNA repair • Substrate-specific protocols • Removal of compounds that inhibit DNA recovery
Sample selection	• Bones, teeth, hair, etc • Dead-end samples (e.g. dinosaurs, amber) DNA remaining in the sample	• Increased power to explore multi-organism substrates, e.g. soil, ice	• Optimization for best-preserved substrates, e.g. hair, specific bones	• Wider taxonomic and geographic range of samples • Greater diversity of substrates • Development of methods for long-term preservation

Fig. 1. Improvements in ancient DNA recovery through time. The introduction of NGS substantially increased the amount of DNA that could be targeted in a single experiment, and more recent methodological advances have resulted in increasingly efficient DNA extraction and library preparation. Paleogenomics will

always be limited by the amount of DNA that survives in a given sample; future advances will stem from continued improvements in DNA recovery efficiency, as well as from technical advances in sequencing, such as single-molecule sequencing, which will allow better characterization of surviving fragments of DNA.

from bones from Vindija Cave in Croatia that contained only 1 to 5% endogenous DNA. This was quickly followed by a 1.9-fold coverage genome from a hominin from Denisova cave (30) and an 11-fold coverage genome from an Australian aborigine (53). The Denisova genome was later improved to 30-fold coverage (28) thanks to very high (~70%) endogenous DNA content and a new, more efficient method to prepare sequencing libraries (54). Recently, a ~50-fold coverage Neandertal paleogenome was recovered from another extremely well preserved bone with a high (~70%) endogenous content, also from a cave in the Altai Mountains of Siberia (31).

Analyses of these paleogenomes revealed several episodes of admixture between hominin lineages during recent evolutionary history. For example, 1 to 4% of the genomes of all modern humans except sub-Saharan Africans is derived from admixture from Neandertals (29, 31). This

finding remains controversial; ancient population structure in the African population ancestral to humans and Neandertals has been proposed as an alternative explanation [e.g., (55–57)]. Analyses of the Denisovan paleogenome convincingly support the admixture model, however. Although the Denisovan mitochondrial genome is distantly related to that of both humans and Neandertals (58), analysis of the Denisovan nuclear genome shows that Denisovans and Neandertals are sister groups with respect to humans. Thus, they are likely descended from the same original hominin group (30, 59). If ancient population structure in Africa were to explain the sharing of alleles between the Vindija Neandertals and modern Eurasians, the Denisova genome should show the same pattern of allele sharing. However, there is no evidence of allele sharing between Denisovans and modern Europeans or East Asians (30). Instead, the Denisova genome shares a number of

rare polymorphisms (around 5 to 7% of the genome) with modern Australian and Melanesian populations (28, 53, 60).

Paleogenomes have also been used to learn specific details about an individual or population. For example, the high-coverage Altai Neandertal paleogenome revealed that inbreeding among close relatives was common in Neandertal population history (31). Within modern humans, paleogenomic analyses have confirmed that the Saqqaq culture represented a different migration from that which later established Inuit populations in Greenland (51), and that Australian aborigines arrived in Australia during a wave of human dispersals before divergence between modern Europeans and Asians (53). At the level of the individual, analyses of a paleogenome of the 5300-year-old Tyrolean Iceman (61) showed that his closest genetic affiliations were with modern Sardinians, even though his remains were recovered from the

Table 1. Paleogenomic and partial paleogenomic data sets generated using NGS and used for genome-scale evolutionary inference, as of December 2013. n/r, not reported; n/a, not applicable; SNP, single-nucleotide polymorphism; indels, insertions and deletions.

Lineage	Common name	Reference	Age (years ago)	Endogenous (%)	Coverage	Average read length
<i>Mammuthus primigenius</i>	Mammoth	(46)	28,000	45.4	Partial: 13 Mb	89
		(65)	18,500*	90	<1×	93
<i>Myotragus balearicus</i>	<i>Myotragus</i>	(41)	6,000	0.27	Partial: 16 kb	60
<i>Homo sapiens</i>	Human	(51)	4,000	84	20×	55
		(53)	100	61	11×	69
		(61)	5,300	77.6	7.6×	n/r
		(87)	7,000 (2 bones)	1.7 and 2.3	Partial: 6 and 1 Mb	75 and 60
		(62)	5,000 (4 bones)	2.4–6.3	Partial: 0.6–1.6 Mb	55
		(88)	~2000 (5 mummies)	~0–21	Partial: up to 1.5 Mb†	100
<i>Homo</i>	Neandertal/ Denisovan	(89)	24,000 and 17,000	17.1 and 0.8	1× and 0.1×	84 and 80
		(29)	3 bones: 38,300, 44,500, undated	<5	1.3×	47
		(30)	74–82,000†	70	1.9×	58, 74
		(28)	74–82,000†	70	30×	n/r
		(31)	~50,000 (Altai) and 60,000–70,000 (Caucasus)	~70 and ~4	52× and 0.5×	71–99 and 47
<i>Ursus maritimus</i>	Polar bear	(63)	120,000	~0.8	0.4×	123
		(64)	43	86	4.3×	122
<i>Gossypium</i>	Cotton	(32)	50,000–3,750 (4 samples)	45.7–95.1	Partial: 16 Mb	37–73
<i>Yersinia pestis</i>	Plague	(33)	663	n/r	30×	56
<i>Mycobacterium tuberculosis</i>	TB	(35)	~150	n/r	Partial: 218 SNPs, 10 indels, 2 repeats	n/r
<i>Equus</i>	Horse	(18)	38,500 and ~700,000 (2 bones)	57.4 and 0.5–4.2	1.78× and 1.12×	n/r and 60
<i>Phytophthora infestans</i>	Irish potato famine	(36)	123–168 (5 samples)	0.4–2.6	3–22×	52–79
		(38)	117–168 (11 samples)	1.0–20	~0–25×	50–85
<i>Mycobacterium leprae</i>	Leprosy	(37)	~1075–700 (7 samples)	0.2–40	5–105	49–109

*Age provided is for the sample selected for the deepest sequencing.
and average number of reads of 150,000.

†Estimated using the “branch shortening” method (28).

‡Estimate based on reported average read length of 100 bp

European Alps. A partial paleogenome from 5000-year-old remains of a human farmer from Gotland, southeastern Sweden, also revealed close affiliations with living southern Europeans and not with 5000-year-old hunter-gatherers from Gotland (62). Together with the Iceman's genome, these data provide evidence that the spread of agriculture across Europe involved the movement of people and not only ideas.

Beyond Hominins and the Future of Paleogenomics

As of October 2013, the only vertebrate lineages other than hominins for which a >1-fold coverage

paleogenome is published are polar bears (*Ursus maritimus*) (63, 64) and horses (*Equus*) (18). A partial mammoth genome has been published (65), but despite the excellent biomolecular preservation of permafrost-preserved mammoth remains, no high-coverage genome yet exists. The small number of paleogenomes is at least partly due to the paucity of fossils with high proportions of endogenous DNA. Some substrates, such as hair, contain higher fractions of endogenous versus environmental DNA than do others, but hair is uncommon in the fossil record. Although there is presently no widely implemented method to predict endogenous DNA

content, quantitative PCR can estimate relative abundance of environmental versus endogenous DNA (66). Sequencing pooled, barcoded libraries to low coverage can also estimate the quality of each library at low cost. Recently, progress has been made in both ancient DNA isolation and target enrichment. For example, a new extraction protocol increases recovery of the shortest DNA fragments (17) and, consequently, may enrich for endogenous DNA, which tends to be more fragmented than environmental and other contaminants. Also, a new method for preparing genomic libraries retains single-stranded, as well as double-stranded, molecules (54).

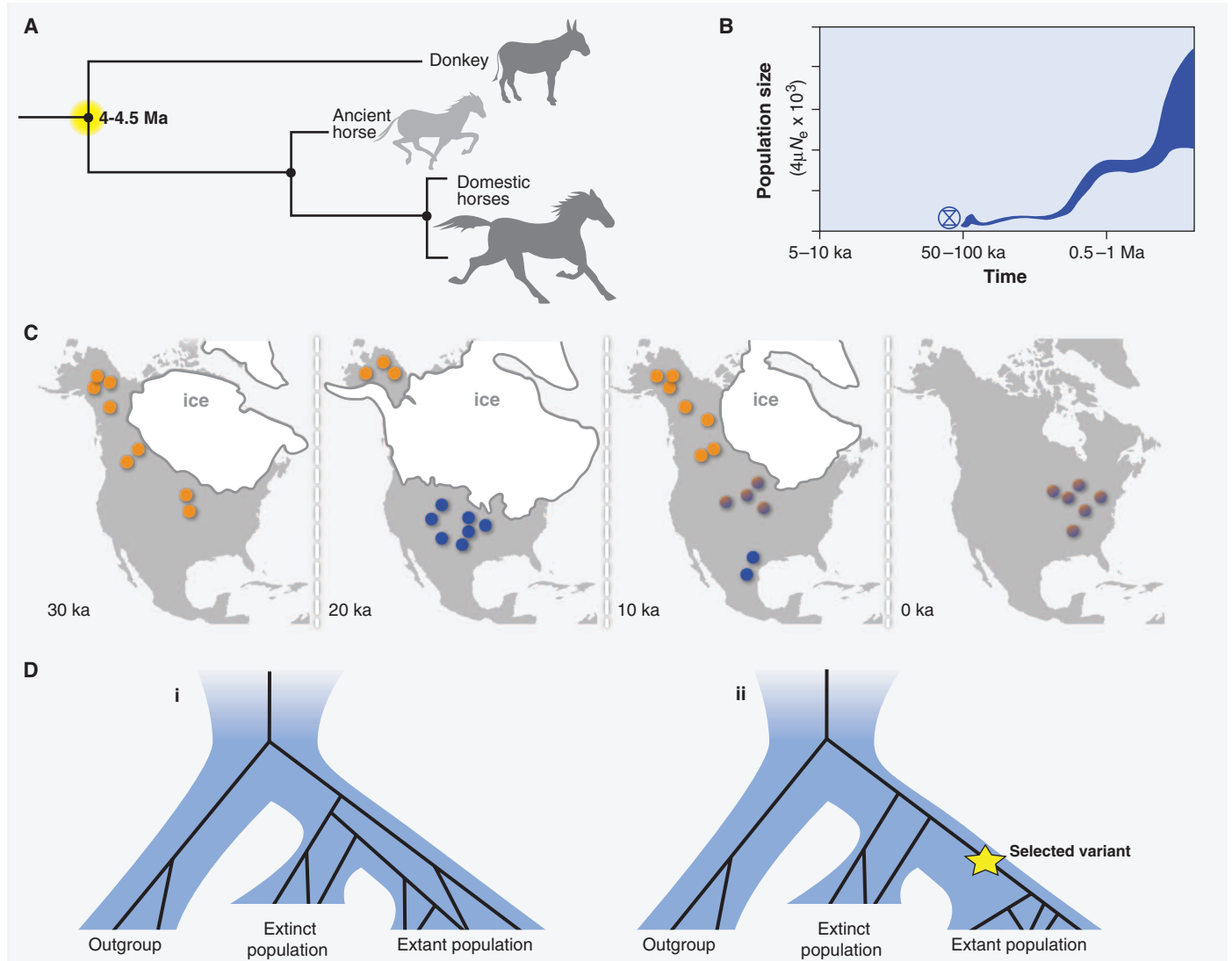


Fig. 2. Insights made possible through the analysis of paleogenomes. (A) Paleogenomes can be used both to resolve evolutionary relationships and provide a source calibration for a molecular clock: Multiple genome alignments of horses including a 700,000-year-old paleogenome pushed back estimates of the divergence among *Equus* to more than 4 Ma (18). (B) High-coverage paleogenomes can be used to infer complex demographic histories of an extinct lineage: The 30× Denisova genome was used to infer the size of the Denisova population through time (28). ka, thousands of years ago. (C) A simulated data set describing how paleogenomic data can reveal the effect of environmental change on genetic diversity. A previously widespread popu-

lation (orange circles) becomes subdivided into two isolated populations (orange and blue) during the glacial maximum (~20 ka), when ice sheets block dispersal between the north and south. As the ice recedes, both populations expand into the deglaciated area, resulting in a hybrid zone (shaded circles). Only admixed individuals survive to the present day. (D) Comparison between loci makes it possible to distinguish regions of the genome or phenotypes that (i) are evolving neutrally versus those that (ii) have undergone a recent selective sweep. Based on comparison with the Neandertal genome, 4235 genomic regions >25 kb in length were identified as having swept to fixation in modern humans (28).

DNA hybridization capture methods also aim to enrich genomic libraries for endogenous relative to environmental DNA. Large-scale enrichment approaches rely on a process of selective hybridization, whereby synthesized bait molecules representing targeted regions hybridize with and immobilize ancient DNA sequences in the library, and anything that does not hybridize is washed away (67). These methods have targeted genomic DNA from hominins (42, 68) and maize (69); mitochondrial genomes of archaic and modern humans (42, 44, 58, 70, 71), horses (18), a cave bear (17), and the oldest putative dog remains (72); and DNA from multiple pathogenic organisms (33–35, 37). One potential complication of whole-genome enrichment is high-copy number sequences, which tend to dominate enriched libraries (69). Nonetheless, capture-enrichment methods have increased the range of samples useful for paleogenomic research and remain a promising area of research.

Interpreting Paleogenomes

Although nonhuman and pathogenic organisms represent a major area of growth in paleogenomics, most nonhuman taxa lack high-quality, annotated, reference genomes. This presents a challenge to genome assembly and limits biological insight. For example, the mammoth paleogenomic data confirmed a slower evolutionary rate among elephants than among hominids (65), but this was described previously from an analysis of mitochondrial genomes (73). Also, the main insight obtained from the ancient horse paleogenome was that the genus *Equus* began to diverge 4 to 4.5 million years ago (Ma), much older than previous estimates (18). Assembling and interpreting paleogenomes will undoubtedly become simpler as more genomes are produced, in particular genomes from taxonomically diverse organisms. As coverage depth increases, it will also become possible to perform analyses that rely on accurate

estimates of heterozygosity, such as estimates of changes in population size through time (27).

Demographic inference and admixture analysis are not the only applications of paleogenomes. Paleogenomes whose ages are well constrained may be useful to calibrate a molecular clock or to investigate genome stability—for example, by tracing movements of transposable elements through time (32). Multiple paleogenomes of the same species will enable inference of changes in selection pressure over time, allowing direct observation of Darwinian evolution. For example, as a reaction to potato blight, plant breeders introduced genes from wild relatives into the potato genome, which provided resistance to infection by the fungus *Phytophthora infestans*. In response, *P. infestans* evolved new effector protein alleles that enabled them to infect resistant plants. *P. infestans* paleogenomes isolated from historic specimens were missing these new alleles (36, 38). Similarly, by observation of genomic differences that accumulate through time, paleogenomes could provide a means to discover domestication-associated genes (74), particularly where comparison between wild and domestic genotypes is not possible, either because the wild form is extinct (e.g., European cattle) (75) or because of relatively recent interbreeding between the wild and domestic forms (e.g., pigs) (76).

Understanding how extinct organisms differed from living organisms remains another major objective of paleogenomics. Linking genotype to phenotype has been possible by using PCR (77, 78); however, few insights have been gained thus far from paleogenomes. Lists of genes that may influence phenotype, generated from positive selection scans, have been published for hominins (29), bears (63), and horses (18). However, these data lack functional verification and remain speculative.

More progress has been made in identifying and describing the function of genes passed into

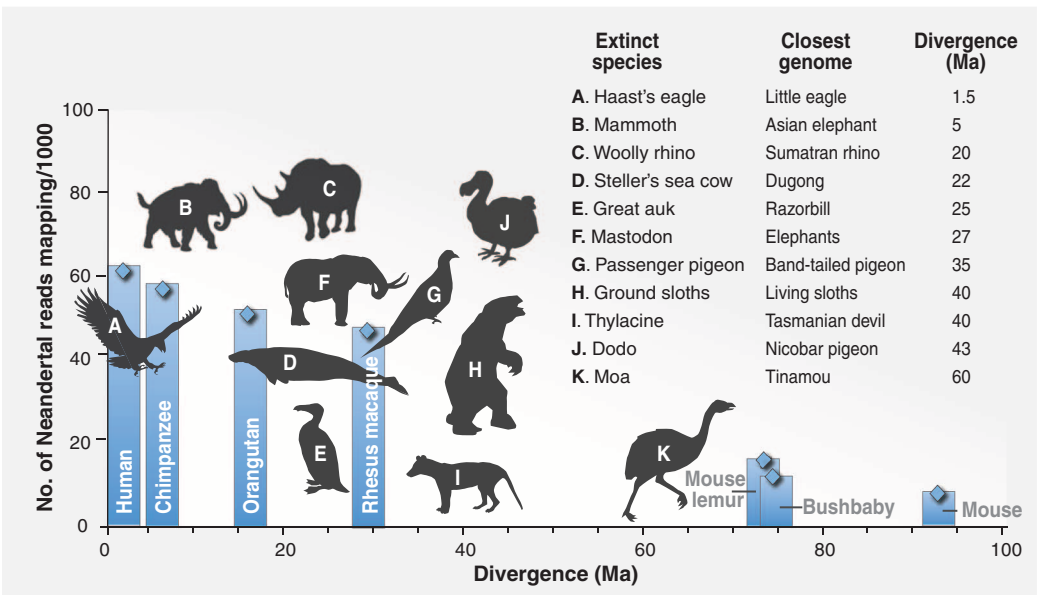
the human lineage after admixture with archaic humans. Abi-Rached *et al.* (79) identified a specific human leukocyte antigen (a gene involved in the human immune response) allele that was acquired by humans from Denisovans and has since risen to high frequency in some west Asian populations. Understanding how the archaic version of this gene differs from human versions, and why the archaic version may be increasing in frequency, may shed light on the evolution of the human immune system. Interestingly, alleles for two other immune-related genes, *OAS* and *STAT2*, have also been identified as having introgressed into modern humans from Neandertals and Denisovans (80, 81).

Looking Ahead

As methods to isolate and sequence endogenous ancient DNA continue to improve, the next few years will almost certainly see an explosion in the taxonomic diversity, number, and temporal range of published paleogenomes. Although the *de novo* assembly of most paleogenomes will remain limited by the short fragment length of ancient genome (82), increasingly evolutionarily diverse genomes from living organisms will provide scaffolds against which most paleogenomes can be assembled (Fig. 3).

A major goal of genomics is to infer function directly from a genome. Although it is not possible to observe many ancient phenotypes, it may be possible to recover epigenetic information from some paleogenomic data sets (83); additional work in this very new area will reveal how useful such epigenetic information will be. Improved annotation of modern genomes will also greatly facilitate the analysis and interpretation of paleogenomes. Better integration with other fields of research, including developmental and synthetic biology and biochemistry, will no doubt facilitate achieving these goals. Although paleogenomes are not necessary to understand how a genome

Fig. 3. The relationship between evolutionary distance and the utility of using an extant taxon as a reference for paleogenome assembly. Increasing evolutionary distance results in a rapid decrease in the proportion of reads mappable to the reference genome (blue bars) (82). We selected 11 species for which obtaining a paleogenome is feasible on the basis of known DNA preservation and plot them against approximate divergence from their closest living relative (x axis). Apart from the moa, most species have a living relative that is diverged by no more than ~50 Ma, suggesting that it should in principle be possible to use their genomes as references for assembling paleogenomes.



encodes an organism, genomic data from extinct lineages will reveal extinct alleles, such as the changes observed in mammoth hemoglobin that appear to have provided an adaptive advantage to elephantids in cold climates (84). Finally, although deextinction remains a controversial topic with many barriers to success (85), a multidisciplinary approach may make it possible to revive extinct phenotypes (86), suggesting that at least some aspects of extinction may not be forever.

Perhaps most importantly, the last few years of paleogenomic research have revealed that the ancient DNA community may have been overcautious with regard to the time scale and range of substrates suitable for analysis. We have learned, for example, that with a conscientious approach to avoiding contamination, it is possible to generate high-quality ancient human genomes. Paleogenomes isolated from pathogenic organisms have confirmed that pathogen DNA survives in the fossil record. A 700,000-year old horse genome (18) and >300,000-year-old mitochondrial genomes from a cave bear (17) and a hominin (71), both from bones preserved in Spanish caves, indicate that DNA preservation extends further back in time and across a wider range of environments than has been generally assumed. Although in many cases individual specimens will continue to yield key information—for example, about demography or paleoecology—the next phase of paleogenomic inference is likely to come from population-level data sets, which will provide a means to explore adaptive evolution directly through time. As the number and range of published paleogenomes grows, paleogenomics is poised to play an increasingly important role in improving our understanding of evolutionary processes over the short and medium term.

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Acknowledgments: We thank J. Cahill for providing coverage statistics for ancient polar bears, and apologize in advance if any studies were inadvertently omitted from Table 1. We thank R. E. Green for helpful comments on earlier drafts of the manuscript.

[10.1126/science.1236573](https://doi.org/10.1126/science.1236573)

INTRODUCTION

Habitability, Taphonomy, and the Search for Organic Carbon on Mars

THE QUEST FOR EXTRATERRESTRIAL LIFE IS AN OLD AND INEVITABLE ambition of modern science. In its practical implementation, the core challenge is to reduce this grand vision into bite-sized pieces of research. In the search for organic remnants of past life, it is enormously helpful to have a paradigm to guide exploration. This begins with assessing habitability: Was the former environment supportive of life? If so, was it also conducive to preservation of organism remains, specifically large organic molecules?

The 2004 arrival of the Mars Exploration Rovers (MERs) Spirit and Opportunity provided a chance to investigate ancient aqueous environments in situ and deduce their likelihood of supporting life. Initial results confirmed what data from orbiters had long suggested—that diverse aqueous environments existed on the surface of Mars billions of years ago. The success of the MERs led to the development of the Mars Science Laboratory (MSL) mission. The Curiosity rover landed at Gale crater in 2012 and was designed to specifically test whether ancient aqueous environments had also been habitable. In addition to water, did these ancient environments also record evidence for the chemical building blocks of life (C, H, N, O, P, S), as well as chemical and/or mineralogic evidence for redox gradients that would have enabled microbial metabolism, such as chemoautotrophy? Curiosity also has the capability to detect organic carbon, but it is not equipped for life detection.

Five articles presented in full in the online edition of *Science* (www.sciencemag.org/extra/curiosity), with abstracts in print (pp. 387–389), describe the detection at Gale crater of a system of ancient environments (including streams, lakes, and groundwater networks) that would have been habitable by chemoautotrophic microorganisms. The Hesperian age (<~3.7 billion years) rocks mentioned in these articles are at the young end of the spectrum of ancient martian aqueous environments. Yet, a sixth article details a more ancient and also potentially habitable environment detected in Noachian age (>~3.7 billion years) rocks at Meridiani Planum. A seventh article describes the present radiation environment on the surface of Mars at Gale crater and its influence on the preservation of organic compounds in rocks.

Opportunity landed at Meridiani Planum on 25 January 2004. Coincident with the 10th anniversary of this landing, Arvidson *et al.*

report the detection of an ancient clay-forming, subsurface aqueous environment at Endeavour crater, Meridiani Planum. Though Opportunity does not have the ability to directly detect C or N, it has been able to establish that several of the other key factors that allow for the identification of a formerly habitable environment were in place. This potentially habitable environment stratigraphically underlies and is considerably older than the rocks detected earlier in the mission that represent acidic, hypersaline environments that would have challenged even the hardest extremophiles. The presence of both Fe⁺³- and Al-rich smectite clay minerals in rocks on the rim of the Noachian age Endeavour

our impact crater were inferred from the joint use of hyperspectral observations by the Mars Reconnaissance Orbiter and extensive surface observations by the Opportunity rover. The rover was guided tactically by orbiter-based mapping. Extensive leaching and formation of Al-rich smectites occurred in subsurface groundwater fracture systems.

Grotzinger *et al.* show that an ancient habitable environment existed at Yellowknife Bay, Gale crater, where stream waters flowed from the crater rim and pooled in a curvilinear depression at the base of Gale's central mountain to form a lake-stream-groundwater system that might have existed for millions of years. Vaniman *et al.* provide evidence for moderate to neutral pH, as shown by the presence of smectite clay minerals and the absence of acid-environment sulfate minerals, and show that the environment had variable redox states due to the presence of mixed-valence Fe (magnetite) and S (sulfide, sulfate) minerals formed within the sediment and cementing rock. McLennan *et al.* show that lake salinities were low because of the very low concentration of salt in the lake deposits. Elemental data indicate that clays were formed in the lake environment and that minimal weathering of the crater rim occurred, suggesting

that a colder and/or drier climate was prevalent.

Ming *et al.* show that the thermal decomposition of rock powder yielded NO and CO₂, indicating the presence of nitrogen- and carbon-bearing materials. CO₂ may have been generated by either carbonate or organic materials. Concurrent evolution of O₂ and chlorinated hydrocarbons indicates the presence of oxychlorine species. Higher abundances of chlorinated hydrocarbons in the lake mudstones, as



Image taken by the Mars Hand Lens Imager of the John Klein drill hole (1.6 cm in diameter) at Yellowknife Bay reveals gray cuttings of mudstone (ancient lake sediments), rock powder, and interior wall. An array of eight ChemCam laser shot points can be seen. The gray color suggests that reduced, rather than oxidized, chemical compounds and minerals dominated the pore fluid chemistry of the ancient sediment. A cross-cutting network of sulfate-filled fractures indicates later flow of groundwater through fractures after the sediment was lithified.

compared with modern windblown materials, suggest that indigenous martian or meteoritic organic C sources are preserved in the mudstone. However, the possibility of terrestrial background sources brought by the rover itself cannot be excluded.

These results demonstrate that early Mars was habitable, but this does not mean that Mars was inhabited. Even for Earth, it was a formidable challenge to prove that microbial life existed billions of years ago—a discovery that occurred almost 100 years after Darwin predicted it, through the recognition of microfossils preserved in silica (1). The trick was finding a material that could preserve cellular structures. A future mission could do the same for Mars if life had existed there. Curiosity can help now by aiding our understanding of how organic compounds are preserved in rocks, which, in turn, could provide guidance to narrow down where and how to find materials that could preserve fossils as well. However, it is not obvious that much organic matter, of either abiologic or biologic origin, might survive degradational rock-forming and environmental processes. Our expectations are conditioned by our understanding of Earth's earliest record of life, which is very sparse.

Paleontology embraces this challenge of record failure with the subdiscipline of taphonomy, through which we seek to understand the preservation process of materials of potential biologic interest. On Mars, a first step would involve detection of complex organic molecules of either abiotic or biotic origin; the point is that organic molecules are reduced and the planet is generally regarded as oxidizing, and so their preservation requires special conditions. For success, several processes must be optimized. Primary enrichment of organics must first occur, and their destruction should be minimized during the conversion of sediment to rock and by limiting exposure of sampled rocks to ionizing radiation. Of these conditions, the third is the least Earth-like (Earth's thick atmosphere and magnetic field greatly reduce incoming radiation). Curiosity can directly measure both the modern dose of ionizing cosmic radiation and the accumulated dose for the interval of time that ancient rocks have been exposed at the surface of Mars.

Hassler *et al.* quantify the present-day radiation environment on Mars that affects how any organic molecules that might be present in ancient rocks may degrade in the shallow surface (that is, the top few meters). This shallow zone is penetrated by radiation, creating a cascade of atomic and subatomic particles that ionize molecules and atoms in their path. Their measurements over the first year of Curiosity's operations provide an instantaneous sample of radiation dose rates affecting rocks, as well as future astronauts. Extrapolating these rates over geologically important periods of time and merging with modeled radiolysis data yields a predicted 1000-fold decrease in 100-atomic mass unit organic molecules in ~650 million years.

Sediments that were buried and lithified beneath the radiation penetration depth, possibly with organic molecules, would eventually be exhumed by erosion and exposed at the surface. During exhumation, organics would become subject to radiation damage as they entered the upper few meters below the rock-atmosphere interface. The time scale of erosion and exhumation, and thus the duration that any parcel of rock is subjected to ionizing radiation, can be determined by measuring cosmogenically produced noble gas isotopes that accumulate in the rock. ^{36}Ar is produced by the capture of cosmogenic neutrons by Cl, whereas ^3He and ^{21}Ne are produced by spallation reactions on the major rock-forming elements. Farley *et al.* show

that the sampled rocks were exposed on the order of ~80 million years ago, suggesting that preservation of any organics that accumulated in the primary environment was possible, although the signal might have been substantially reduced.

Wind-induced saltation abrasion of the rocks in Yellowknife Bay appears to have been the mechanism responsible for erosion and exhumation of the ancient lake bed sampled by Curiosity. The geomorphic expression of this process is a series of rocky scarps that retreated in the downwind direction. Understanding this process leads to the prediction that rocks closest to the scarps were most recently exhumed and are thus most likely to preserve organics, all other factors being equal. In this manner, the MSL mission has evolved from initially seeking to understand the habitability of ancient Mars to developing predictive models for the taphonomy of martian organic matter. This parallels the previous decade, in which the MER mission turned the corner from a mission dedicated to detecting ancient aqueous environments to one devoted to understanding how to search for that subset of aqueous environments that may also have been habitable.

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10.1126/science.1249944

ABSTRACTS

Ancient Aqueous Environments at Endeavour Crater, Mars

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Opportunity has investigated in detail rocks on the rim of the Noachian age Endeavour crater, where orbital spectral reflectance signatures indicate the presence of Fe³⁺-rich smectites. The signatures are associated with fine-grained, layered rocks containing spherules of diagenetic or impact origin. The layered rocks are overlain by breccias, and both units are cut by calcium sulfate veins precipitated from fluids that circulated after the Endeavour impact. Compositional data for fractures in the layered rocks suggest formation of Al-rich smectites by aqueous leaching. Evidence is thus preserved for water-rock interactions before and after the impact, with aqueous environments of slightly acidic to circum-neutral pH that would have been more favorable for prebiotic chemistry and microorganisms than those recorded by younger sulfate-rich rocks at Meridiani Planum.

>> Read the full article at <http://dx.doi.org/10.1126/science.1248097>

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A Habitable Fluvio-Lacustrine Environment at Yellowknife Bay, Gale Crater, Mars

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The Curiosity rover discovered fine-grained sedimentary rocks, which are inferred to represent an ancient lake and preserve evidence of an environment that would have been suited to support a martian biosphere founded on chemolithoautotrophy. This aqueous environment was characterized by neutral pH, low salinity, and variable redox states of both iron and sulfur species. Carbon, hydrogen, oxygen, sulfur, nitrogen, and phosphorus were measured directly as key biogenic elements; by inference, phosphorus is assumed to have been available. The environment probably had a minimum duration of hundreds to tens of thousands of years. These results highlight the biological viability of fluvial-lacustrine environments in the post-Noachian history of Mars.

>> Read the full article at <http://dx.doi.org/10.1126/science.1242777>

Mineralogy of a Mudstone at Yellowknife Bay, Gale Crater, Mars

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Sedimentary rocks at Yellowknife Bay (Gale crater) on Mars include mudstone sampled by the Curiosity rover. The samples, John Klein and Cumberland, contain detrital basaltic minerals, calcium sulfates, iron oxide or hydroxides, iron sulfides, amorphous material, and trioctahedral smectites. The John Klein smectite has basal spacing of ~10 angstroms, indicating little inter-layer hydration. The Cumberland smectite has basal spacing at both ~13.2 and ~10 angstroms. The larger spacing suggests a partially chloritized inter-layer or interlayer magnesium or calcium facilitating H₂O retention. Basaltic minerals in the mudstone are similar to those in nearby eolian deposits. However, the mudstone has far less Fe-forsterite, possibly lost with formation of smectite plus magnetite. Late Noachian/Early Hesperian or younger age indicates that clay mineral formation on Mars extended beyond Noachian time.

>> Read the full article at <http://dx.doi.org/10.1126/science.1243480>

Elemental Geochemistry of Sedimentary Rocks at Yellowknife Bay, Gale Crater, Mars

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Sedimentary rocks examined by the Curiosity rover at Yellowknife Bay, Mars, were derived from sources that evolved from an approximately average martian crustal composition to one influenced by alkaline basalts. No evidence of chemical weathering is preserved, indicating arid, possibly cold, paleoclimates and rapid erosion and deposition. The absence of predicted geochemical variations indicates that magnetite and phyllosilicates formed by diagenesis under low-temperature, circumneutral pH, rock-dominated aqueous conditions. Analyses of diagenetic features (including concretions, raised ridges, and fractures) at high spatial resolution indicate that they are composed of iron- and halogen-rich components, magnesium-iron-chlorine-rich components, and hydrated calcium sulfates, respectively. Composition of a cross-cutting dike-like feature is consistent with sedimentary intrusion. The geochemistry of these sedimentary rocks provides further evidence for diverse depositional and diagenetic sedimentary environments during the early history of Mars.

>> Read the full article at <http://dx.doi.org/10.1126/science.1244734>

Volatile and Organic Compositions of Sedimentary Rocks in Yellowknife Bay, Gale Crater, Mars

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H₂O, CO₂, SO₂, O₂, H₂, H₂S, HCl, chlorinated hydrocarbons, NO, and other trace gases were evolved during pyrolysis of two mudstone samples acquired by the Curiosity rover at Yellowknife Bay within Gale crater, Mars. H₂O/OH-bearing phases included 2:1 phyllosilicate(s), bassanite, akaganeite, and amorphous materials. Thermal decomposition of carbonates and combustion of organic materials are candidate sources for the CO₂. Concurrent evolution of O₂ and chlorinated hydrocarbons suggests the presence of oxychlorine phase(s). Sulfides are likely sources for sulfur-bearing species. Higher abundances of chlorinated hydrocarbons in the mudstone compared with Rocknest windblown materials previously analyzed by Curiosity suggest that indigenous martian or meteoritic organic carbon sources may be preserved in the mudstone; however, the carbon source for the chlorinated hydrocarbons is not definitively of martian origin.

>> Read the full article at <http://dx.doi.org/10.1126/science.1245267>

Mars' Surface Radiation Environment Measured with the Mars Science Laboratory's Curiosity Rover

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The Radiation Assessment Detector (RAD) on the Mars Science Laboratory's Curiosity rover began making detailed measurements of the cosmic ray and energetic particle radiation environment on the surface of Mars on 7 August 2012. We report and discuss measurements of the absorbed dose and dose equivalent from galactic cosmic rays and solar energetic particles on the martian surface for ~300 days of observations during the current solar maximum. These measurements provide insight into the radiation hazards associated with a human mission to the surface of Mars and provide an anchor point with which to model the subsurface radiation environment, with implications for microbial survival times of any possible extant or past life, as well as for the preservation of potential organic biosignatures of the ancient martian environment.

>> Read the full article at <http://dx.doi.org/10.1126/science.1244797>

In Situ Radiometric and Exposure Age Dating of the Martian Surface

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We determined radiogenic and cosmogenic noble gases in a mudstone on the floor of Gale crater. A K-Ar age of 4.21 ± 0.35 billion years represents a mixture of detrital and authigenic components and confirms the expected antiquity of rocks comprising the crater rim. Cosmic-ray-produced ³He, ²¹Ne, and ³⁶Ar yield concordant surface exposure ages of 78 ± 30 million years. Surface exposure occurred mainly in the present geomorphic setting rather than during primary erosion and transport. Our observations are consistent with mudstone deposition shortly after the Gale impact or possibly in a later event of rapid erosion and deposition. The mudstone remained buried until recent exposure by wind-driven scarp retreat. Sedimentary rocks exposed by this mechanism may thus offer the best potential for organic biomarker preservation against destruction by cosmic radiation.

>> Read the full article at <http://dx.doi.org/10.1126/science.1247166>



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A Habitable Fluvio-Lacustrine Environment at Yellowknife Bay, Gale Crater, Mars

J. P. Grotzinger *et al.*

Science **343**, (2014);

DOI: 10.1126/science.1242777

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A Habitable Fluvio-Lacustrine Environment at Yellowknife Bay, Gale Crater, Mars

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The Curiosity rover discovered fine-grained sedimentary rocks, which are inferred to represent an ancient lake and preserve evidence of an environment that would have been suited to support a martian biosphere founded on chemolithoautotrophy. This aqueous environment was characterized by neutral pH, low salinity, and variable redox states of both iron and sulfur species. Carbon, hydrogen, oxygen, sulfur, nitrogen, and phosphorus were measured directly as key biogenic elements; by inference, phosphorus is assumed to have been available. The environment probably had a minimum duration of hundreds to tens of thousands of years. These results highlight the biological viability of fluvial-lacustrine environments in the post-Noachian history of Mars.

The environmental history of early Mars is mostly written in stone. The first few billion years of Mars' geologic history document surface environments considerably different than the surface today, prompting a succession of coordinated surface and orbiter missions over the past two decades aimed at ultimately determining if Mars ever had an early biosphere. Water is the essential ingredient for all life as we understand it; therefore, past missions have sought environments in which water was abundant and possibly long-lived. However, to create an environment that could have been habitable by microorganisms, the presence of water alone is insufficient: A source of energy to fuel microbial metabolism is required, as are the elements carbon, hydrogen, sulfur, nitrogen, and phosphorous (and a host of others at trace levels). The Mars Science Laboratory (MSL) rover Curiosity was designed and built to search for these materials and, thus, potentially delineate one or more habitable environments at the Gale crater landing site. We show here that Gale crater once contained an ancient lake that would have been well suited to support a martian biosphere founded on chemolithoautotrophy.

Exploration for habitable environments is Curiosity's core mission objective. Achieving this goal necessitates a challenging set of science measurements on just the right rocks to extend beyond the search for aqueous environments. To enhance the probability of mission success, we adopted an exploration strategy that

included well-defined secondary objectives in addition to exploring Gale's central mountain, Aeolis Mons (informally known as Mt. Sharp), the mission's primary science target. The selection of the Gale crater field site, where Curiosity landed on 6 August 2012, 5:32 UTC, was based on the recognition that it retained records of multiple and diverse ancient aqueous environments and thus enhanced the potential that one or more of those settings might have provided the combination of factors necessary to sustain a habitable environment (*1*). Another important factor included mapping the landing ellipse ahead of landing so that no matter where the rover touched down, our first drive would take us in the direction of a science target deemed to have the greatest value, as weighed against longer-term objectives and the risk of mobility failure.

Mapping and Geologic Framework for Exploration

Gale crater is 154 km in diameter with a central mountain of stratified rock. Gale straddles the crustal dichotomy boundary, a topographic demarcation between heavily cratered uplands and sparsely cratered lowlands. In the vicinity of Gale, the dichotomy boundary is crossed by numerous incised valley networks suggestive of surface aqueous flows that discharged to the north across the lowlands (*2*). Gale's stratigraphy, mineralogy, and landforms have been well studied from orbit (*3–6*). A broader context for understanding Mt. Sharp has been devel-

oped (*7*), along with Gale's relevance to MSL's goals (*1, 8*). The primary mission objective is to examine the lower foothills of Mt. Sharp (Fig. 1A), located outside of the landing ellipse, where hematitic and hydrated clay- and sulfate-bearing strata were detected from orbit (*5, 9*) and are accessible by the rover (*1*).

The surface mission was planned in advance (*1*) to take into account the drive distance from anywhere in the landing ellipse up to and across the Mt. Sharp foothills, including time for sampling and analyses en route. The landing ellipse is located in the broad valley (Aeolis Palus) between the Gale crater rim and Mt. Sharp (Fig. 1A). Depending on where

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Curiosity touched down, potential secondary science targets within the landing ellipse included the Peace Vallis alluvial (water-deposited) fan lobe; a high-thermal inertia terrain localized downslope from and adjacent to the fan; several fresh craters; and inverted stream channels. In the case of the current study, a secondary target near the landing site became a notable objective, with the decision (10) made after landing to drive in the opposite direction of the Mt. Sharp entry point to sample stratified rocks of relatively high

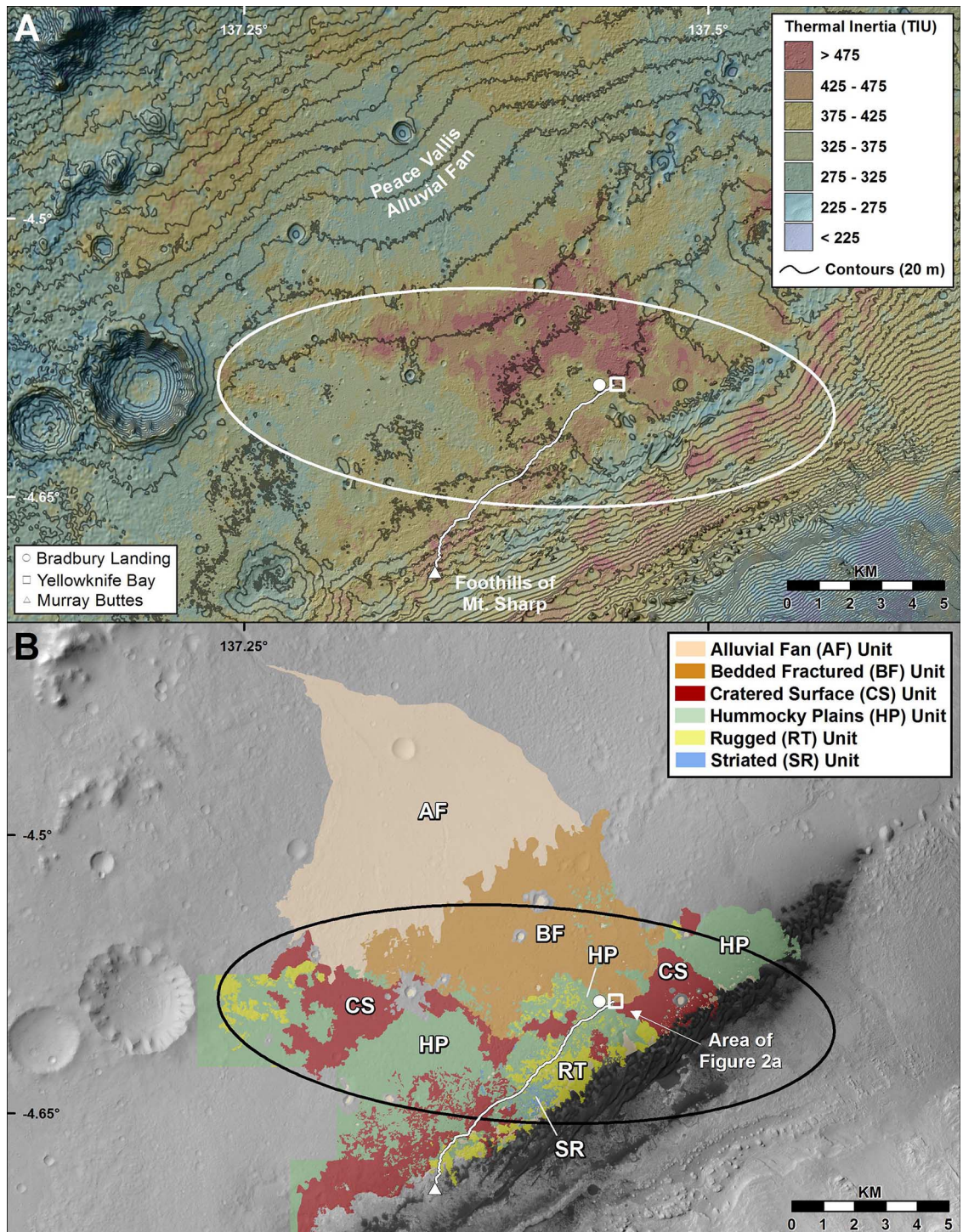
thermal inertia exposed at Yellowknife Bay (11) (Figs. 1A and 2A).

Geologic mapping (12), building on earlier efforts (4), proved critically important to selecting the postlanding drive direction and science targets. This work provided the broader context in which to understand the strata drilled by Curiosity in Yellowknife Bay. The MSL team-generated map (Fig. 1B) reveals six units (13) that are distinguished based on variations in thermal inertia; textural attributes such as the

presence of scarps and lineations consistent with bedding; surface roughness based on shadowing; apparent relative albedo; and patterns of albedo variation such as those indicating fractures, bedding, or mottling. These units include the Peace Vallis alluvial fan (AF); an immediately adjacent and downslope light-toned, bedded, fractured unit (BF); surfaces with relatively high crater densities (CS); tonally smooth but hummocky plains (HP); light-toned topographically variable or rugged terrain (RT); and light-toned

Fig. 1. Regional context maps.

(A) Location of Bradbury landing site and Yellowknife Bay in relation to the topography and thermal inertia of the broad valley between Gale crater's rim and Mt. Sharp. The white ellipse denotes the landing ellipse; the white line represents the intended drive route from Yellowknife Bay to the Mt. Sharp entry point at Murray Buttes. (B) A geological map was constructed based largely on HiRISE image data and was used to demarcate major terrain types for exploration by Curiosity. The MSL Science Team chose to drive the rover to Yellowknife Bay where three terrains intersect in a triple junction. One of these terrains (BF) very closely coincides with higher values of thermal inertia, and it was also recognized that rocks at Yellowknife Bay are downslope of those forming the AF unit, suggesting a genetic link. The black ellipse is the landing ellipse; the white line represents the intended drive route from Yellowknife Bay to the Mt. Sharp entry point. The legend for symbols in (A) also applies to those in (B).



striated rocks (SR). By sol 440 (where 1 sol is a martian day), the RT and SR units had not been examined by Curiosity and are not discussed further. Both are considered as exposures of bedrock based on textures and higher thermal inertia values. The CS unit was inspected only from a distance during the traverses to and from Yellowknife Bay and probably reflects lateral equivalents of bedrock studied by Curiosity, discussed further below.

The HP unit is exposed in the vicinity of Bradbury Landing (14) and has lower values of thermal inertia. It is composed dominantly of loose materials, including clasts interpreted to be impact ejecta, but also displays erosional remnants of underlying bedrock. Analyzed clasts have basaltic, alkalic (K- and Na-rich) compositions (15), and some clasts and soils contain crystals of probable feldspar (16). Windows through this material in the vicinity of Bradbury

Landing, including the excavation zones produced by Curiosity's descent rockets, expose pebble conglomerate bedrock of fluvial (stream-related) origin (17).

The AF and BF units are important because of their intimate spatial association and possible relation to rocks exposed at Yellowknife Bay. The AF unit is defined by the Peace Vallis fan, which extends from the northwest rim of Gale crater (Fig. 1B); it may be the youngest component of a much more extensive alluvial plain that flanks the crater wall (4). The Peace Vallis fan probably formed by routing of eroded crater rim-derived rock fragments and any materials that accumulated there after crater formation, through Peace Vallis followed by downstream spreading and deceleration of flows and deposition of sediments in the downslope direction. The upslope part of the AF unit is marked by smooth mottled surfaces punctuated by slope-

aligned linear ridges with a relief of 0.5 to 2.5 m interpreted as inverted stream channels. Downslope, this unit passes into the widespread BF unit that continues all the way to Yellowknife Bay, where it is coincident with the stratified bedrock exposed there and the terrain that marks the floor of the "bay" (18) itself. Although map relations suggest that the BF unit represents distal fan facies (rock types), these relations do not require that the Yellowknife Bay BF exposures be contemporaneous with the Peace Vallis lobe of the alluvial plain; they could represent an older component of the alluvial plain, separated from the youngest activity by an unconformity (see below). Nevertheless, the close spatial association implies a genetic link, with the BF unit having formed in a probably distal alluvial or even lacustrine (lake-related) setting, consistent with earlier mapping (4).

Geologic units in the interior of Gale crater display a range of relative ages and preserve a substantial record of geologic activity since the crater formed close to the Noachian–Early Hesperian boundary ~3.7 billion years ago (6). The oldest units are probably of Early Hesperian age and likely include the bulk of the alluvial plain that extends downslope of the crater rim. The Peace Vallis alluvial lobe (map unit AF) appears to represent the latest stage of transport and deposition on the alluvial plain.

Our mapping shows that strata exposed at Yellowknife Bay are probably fan, distal fan, or downslope equivalents such as lacustrine deposits. Despite the ambiguity in the relative age of these strata, the fluvial-lacustrine depositional context remains intact, and this is what motivated the MSL Science Team to explore these rocks.

Stratigraphy and Sedimentology of Yellowknife Bay Rocks

Bradbury Landing occurs near the crest of a broad topographic high, several kilometers in width, informally called Bradbury Rise. To the north and east, the rise descends to rocks of the BF unit. After landing, the team-consensus decision was to traverse eastward and downhill to the HP-BF contact, as close as possible to the triple junction where the CS unit also intersects (Fig. 1B). Between Bradbury Landing and Yellowknife Bay, Curiosity crossed a straight-line distance of 445 m and descended 18 m in elevation. Most of this distance was spent traversing the clast-strewn HP unit, with its occasional outcrops of pebble conglomerate facies. Beginning at the Bathurst Inlet outcrop, a succession of strata was encountered with good exposure down to the floor of Yellowknife Bay (Fig. 2A). In ascending order, these strata are informally named the Sheepbed (>1.5 m thick), Gillespie Lake (~2.0 m thick), and Glenelg members (~1.7 m thick); the assemblage of members is known as the Yellowknife Bay formation, and the exposed section is ~5.2 m thick. (Fig. 3 and table S1). Strata are largely decimeter-scale in thickness and comprise a heterogeneous assemblage

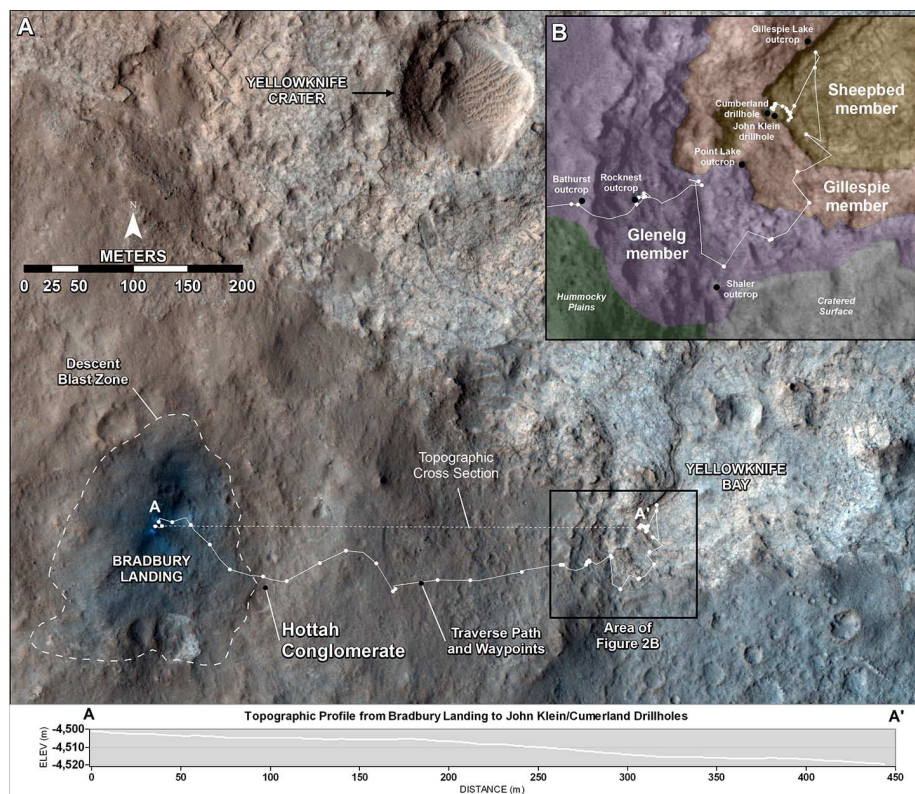


Fig. 2. Local context maps. (A) HiRISE image (PSP_010573_1755) of Bradbury Landing showing Curiosity's position after landing and the path of eventual traverse to Yellowknife Bay, where the John Klein and Cumberland holes were drilled. The area of this image is approximated by the circle and square symbols in Fig. 1B. Yellowknife Bay derives its name from Yellowknife crater, which is located within the broad expanse of the BF map unit. The BF unit probably represents the Yellowknife Bay formation at more regional scales. Cross section (A to A') shows topography from Bradbury Landing to Yellowknife Bay. (B) The inset shows a detailed geological map of the southwest part of Yellowknife Bay. Three map units (HP, CS, BF) intersect just south of the Shaler outcrop. The BF map unit is subdivided into three members (see text). Key outcrops are shown that provided information leading to the development of a stratigraphy shown in Fig. 4. Note that the contact between Sheepbed member and Gillespie Lake member erodes to form a topographic step that is visible from orbit and can be traced for hundreds of meters around Yellowknife Bay. Therefore, it seems likely that much of the Yellowknife Bay topographic depression was created by eolian erosion of the Sheepbed mudstone member (see text). See Fig. 4 for mapped stratigraphic units. Locations of Rocknest (scoop location and rocky outcrops) and John Klein and Cumberland (drill) sampling locations are shown.

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of mostly detrital sedimentary rocks of basaltic bulk composition. Breaks in outcrop continuity due to accumulated loose materials on the slope (Fig. 3) introduce a degree of uncertainty in understanding lateral extent of bedding; the lowest stratigraphic members (Sheepbed and Gillespie Lake) are best exposed around the perimeter of Yellowknife Bay (Fig. 2B). The Glenelg member contains a diverse suite of facies formed of beds with demonstrably less lateral continuity, although most beds can be traced for at least tens of meters.

The basal Sheepbed and Gillespie Lake members are characterized (19) by elemental compositions broadly consistent with a provenance that, on average, is similar to typical martian upper crust (20). In addition, these rocks carry the signal of higher thermal inertia as seen from orbit. Subtle geochemical variations within and between these units mostly reflect minor variations in provenance. A marked change in composition, best reflected by a sharp increase in K_2O contents and K_2O/Al_2O_3 ratios (Fig. 4), takes place higher in the section in the Glenelg member (Bathurst Inlet outcrop), suggesting a fundamental change in the provenance to one dominated by more alkaline igneous rocks (16, 21). This change could result from either propagation of the fluvial system headwaters into an area with more alkaline igneous rocks or a shift to more locally derived volcanic debris, such as reworked volcanic rocks or ash deposits (21). Major element compositions do not indicate substantial chemical weathering in the sediment source area, which implicates a frigid or arid climate, a high-relief source area undergoing rapid erosion, or a combination of these factors (19, 21).

In general, the Yellowknife Bay formation (Fig. 4) is composed of fine-, medium-, and coarse-grained sandstones of basaltic composition. The Shaler outcrop (~125 cm thick) consists of a heterogeneous assemblage of interstratified platy coarser-grained beds comprising sandstones and likely pebbly units, separated by recessive, probably finer-grained intervals. Coarser-grained intervals are characterized by distinct decimeter-scale trough cross-bedding (Fig. 5A). Recessive intervals are marked by downward-tapering cracks that terminate upward at bedding planes and are probably filled with sediment from the overlying bed. The rock termed “Rocknest-3” is very fine-grained, finely laminated sandstone or siltstone with shrinkage cracks (Fig. 5B). Volcanic or impact-related base-surge deposition is excluded by the abundance of decimeter-scale and compound cross-bedding, the absence of stoss-side cross-stratification with preservation only of bedform toesets, and variable cross-bed dip directions. Furthermore, there are hierarchical scales of cross-stratification at the Shaler outcrop, indicating migration of superimposed bedforms of different scales. These attributes are most consistent with a former fluvial environment characterized by bedload and suspended load transport and variable, but net southeast flow directions. However, some eolian bedload transport is suggested by intercalated beds with pinstripe lamination. Most paleocurrent data are restricted to 90° of variability (fig. S1); however, some scatter to almost 180°, which is consistent with migration of three-dimensional (3D) superimposed bedforms (22). The inferred sediment transport direction suggests derivation of sediments from the direction of the Gale crater rim.

A fluvial interpretation is further supported by the tapered, likely sediment-filled cracks that are most easily accounted for by desiccation-induced shrinkage of finer-grained interbeds. These interbeds could represent either fluvial overbank deposits, slack water deposits in the troughs between bedform crests, or intercalated tongues of proximal lacustrine facies.

The Point Lake outcrop (~50 cm thick) is noteworthy for its pervasive centimeter-scale voids (Fig. 5C). These voids could possibly point to a volcanic origin, specifically a vesicular lava flow (23). Alternatively, it is possible that this facies also represents a pebbly debris flow or gas-charged intrusive sedimentary sill (24, 25). Scattered blebs of light-toned material were evaluated as calcium sulfate by ChemCam (19) and appear to fill voids and narrow fractures. Thus, it is possible that the rock could be a sandstone with leached nodular evaporites (26).

The Sheepbed and stratigraphically overlying Gillespie Lake members (Figs. 3 and 4) make up the exposed base of the Yellowknife Bay formation, and their contact has eroded to form a decimeter-scale distinctive topographic step observable in High Resolution Imaging Science Experiment (HiRISE) orbiter image data (Fig. 2A). The Gillespie Lake member consists of amalgamated, sheetlike sandstones. Bedding generally has a massive appearance, though poorly defined cross-bedding is present. The basal bed of the Gillespie Lake member is formed of poorly sorted, angular to moderately well-rounded, medium to very coarse sand of bulk basaltic composition (Fig. 5D). Some of the largest grains have translucent luster. Very little residual porosity between grains is evident, which suggests

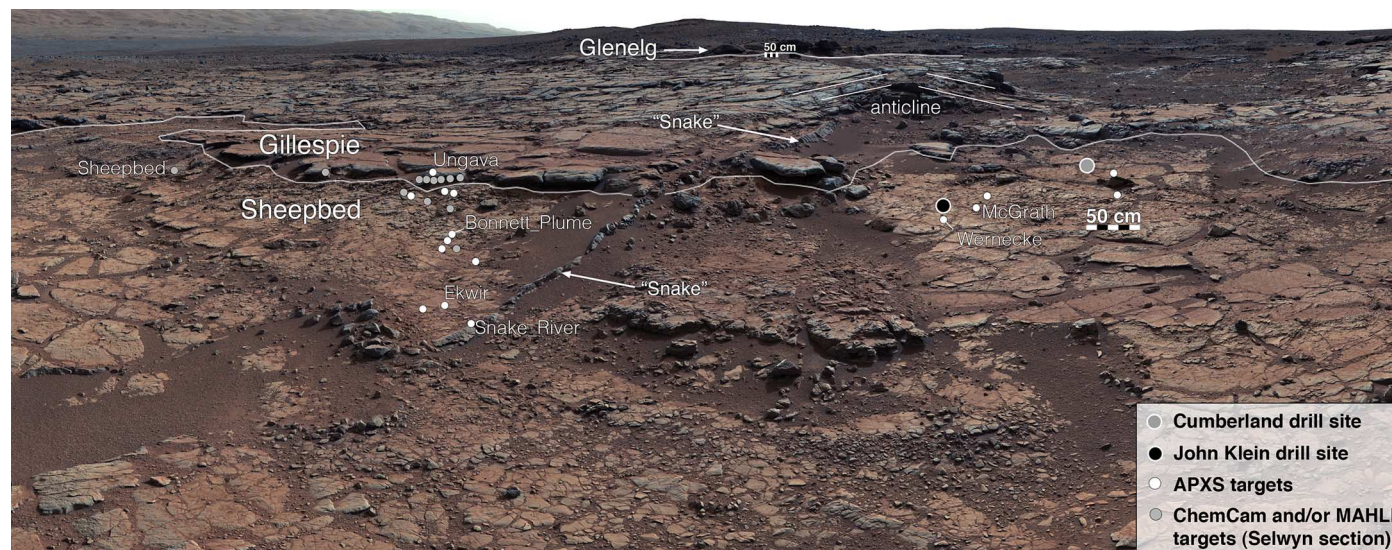


Fig. 3. Mastcam mosaic of Yellowknife Bay formation. View from the base of the exposed section up through Sheepbed, Gillespie Lake, and basal Glenelg members. Locations of drill holes and APXS measurements are shown. High density of APXS measurements between Snake River and Ungava targets delineates the route of the Selwyn reference section. Note the “snake” feature cutting up through the section and terminating in a small anticline. White dots represent combined APXS, MAHLI, and ChemCam measurements; gray dots

denote ChemCam or MAHLI only. Both scale bars are 50 cm long with 10-cm-spaced white and black sections. The lower scale bar is ~8 m away from Curiosity; the upper scale bar is ~30 m away. The 111-image mosaic was acquired on sol 137, by M-34 sequence 818. This figure shows only a small portion of the full mosaic. The foothills of Mt. Sharp are visible in the distance, upper left, southwest of camera position. For mosaic image identification numbers (IDs), see (31).

a high degree of cementation; what porosity does exist appears as sparse, millimeter-scale irregular vugs (void spaces). This basal bed has a sharp, erosional base that shows centimeter-scale scouring into the underlying Sheepbed member mudstones and appears to show lateral variation in its thickness. The poor sorting, massive bedding, and variability of grain shape in the Gillespie Lake sandstones are most consistent with fluvial transport and deposition. The sheetlike geometry of the beds, extending over hundreds of meters,

and the absence of well-developed channel bodies may indicate deposition as distal fan lobes, as documented in examples from ancient alluvial fans on Earth (27).

The pebble conglomerate outcrops distributed across the HP map unit have an uncertain stratigraphic relation with respect to the Yellowknife Bay formation. On one hand, the conglomerate exposures occur at a topographically high level above flat-lying rocks of the Yellowknife Bay formation; the simplest interpretation would in-

voke superposition and, therefore, a younger age for the conglomerate than the Yellowknife Bay formation. However, regional mapping allows for, but does not require, an alternative possibility in which flat-lying Yellowknife Bay rocks unconformably onlap older rocks that underlie the HP map unit; in this interpretation, the conglomerate is relatively older. Unfortunately, outcrop between Yellowknife Bay rocks and the nearest conglomerate is very poor, and it is not possible to observe a contact. Therefore, both

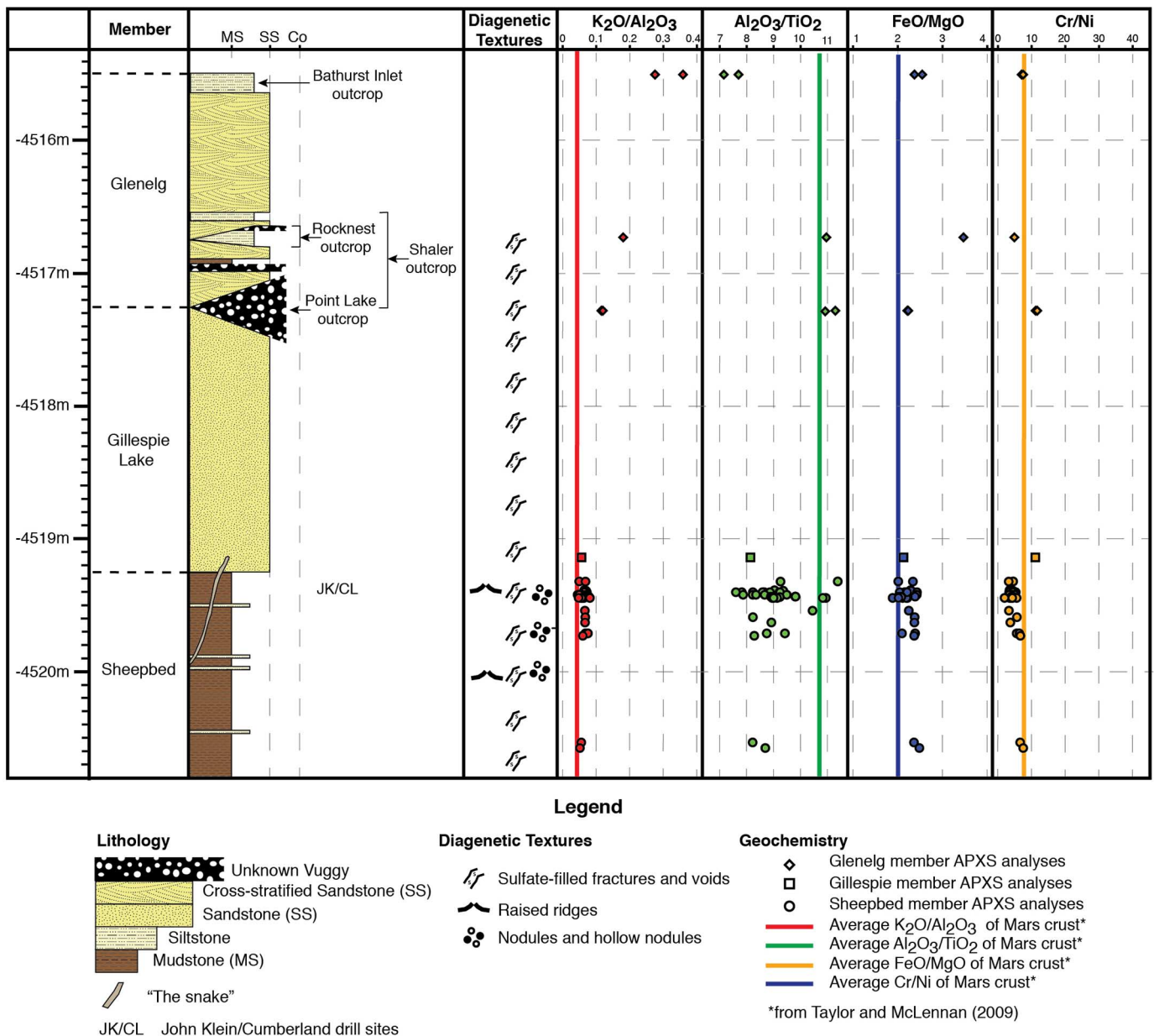


Fig. 4. Stratigraphy of the Yellowknife Bay formation plotted with elemental and oxide ratios measured by the Curiosity APXS instrument. The Yellowknife Bay section was split into three members distinguished by lithological properties (facies, textures), chemical composition, and attributes observed from orbit by HiRISE. The stratigraphic column highlights differences in grain size between the Yellowknife Bay members and the presence

of stratification styles; primary textures; and diagenetic textures including nodules, hollow nodules, raised ridges, sulfate-filled fractures, and vugs. APXS analyses are plotted as ratios to highlight compositional trends throughout the Yellowknife Bay formation. Average Mars crustal composition for each ratio (colored vertical lines) (20) is plotted for comparison to Curiosity APXS measurements.

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options are feasible, but in either case, these rocks all belong to the same genetically related suite of fluvial-lacustrine sediments derived from the crater rim.

Sheepbed Mudstone Member

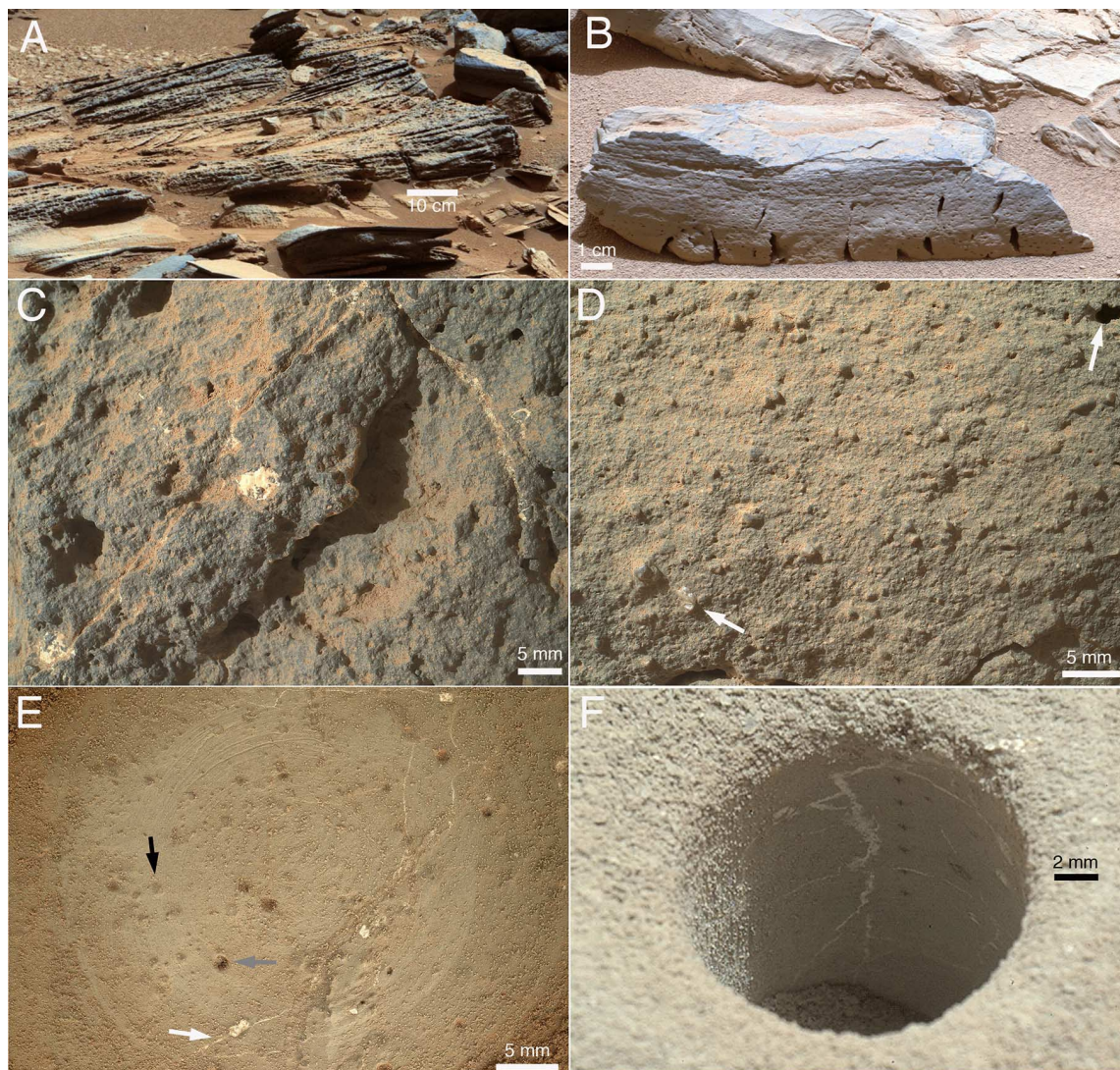
At a minimum, the Sheepbed member extends laterally across the width of the outcrop belt traversed by the rover: ~60 m. The Sheepbed member closely coincides with the BF map unit and from orbit is highlighted by its light tone and extensive meter- to decameter-scale fracture network. Tracing of the distinctive erosional pro-

file of the Sheepbed–Gillespie Lake contact shows that the Sheepbed member probably extends around the full width of Yellowknife Bay and covers a minimum area of ~4 km². However, we presume the Sheepbed member extends beneath the overlying rocks of the Gillespie Lake member; therefore, these are highly conservative minimum estimates. A reference section localized near the Selwyn target (Fig. 3) yields a thickness of 1.5 m for the member. This is a minimum thickness because its base is not exposed.

A detailed traverse (Selwyn reference section) using Mastcam, ChemCam, Alpha Particle

X-ray Spectrometer (APXS), and Mars Hand Lens Imager (MAHLI) measurements was conducted across the Sheepbed member for the purpose of providing context for the first samples obtained by drilling (Figs. 3 and 4). Observations revealed a patchy distribution of diagenetic (postdepositional) features, including nodules and veins, as discussed below. Mineralogic data show that the Sheepbed member has a substantial quantity of saponitic smectite clay minerals (~20%), probably formed by aqueous alteration of olivine (28). Geochemical results (19) show that both Sheepbed and Gillespie

Fig. 5. Sedimentary rocks of the Yellowknife Bay formation. (A) Shaler outcrop shows a variety of facies, including compound cross-bedding. The image was white-balanced and acquired by Mastcam-100 on sol 120. Image ID is 0120MR00075201302-00690E01_DXXX. (B) The Rocknest-3 rock, which represents the middle part of the Glenelg member, is fine-grained with planar lamination, shrinkage cracks, and millimeter-scale voids (well developed in the lower third section of the rock). This image is a subset of a mosaic of images that were white-balanced and acquired with Mastcam-100 on sol 59. Image IDs, as part of SEQID mcam00270, are 0059MR00027000001031-41E01_DXXX, 0059MR000-2700010103142E01_DXXX, 0059MR00027000201031-43E02_DXXX, and 0059MR-0002700030103144E01_DXXX. (C) Vuggy, medium-grained texture in the Point Lake outcrop (“Measles Point” target), middle of the Point Lake member. Note the thin fractures filled with light-toned material, interpreted as sulfate. ChemCam data show that white blebs, such as the one in the center, are composed of calcium sulfate. This focus merge product was acquired by MAHLI on sol 303, ID 0303MH0002900000103786R00. (D) Medium- to coarse-grained sandstone at the Gillespie Lake outcrop (see Fig. 2B), basal bed of the Gillespie Lake member. This bed is composed of poorly sorted, moderately well-rounded, dark to light grains, some of which have glassy luster (lower left arrow). A small amount of vuggy porosity is evident (upper right arrow), but otherwise the rock appears well cemented. The image was acquired by MAHLI on sol 132, ID 0132MH0001580010101221E01. (E) Mudstone in Sheepbed member, “Wernecke target” (see Fig. 3). The rock has been brushed, revealing homogeneous, fine-grained texture. Outlines of nodules (black arrow), void



spaces (gray arrow), and sulfate-filled voids can be seen, and only those voids connected by hairline fractures (white arrow) have been filled with sulfates. Note the surface scoring by brush bristles, indicating softness of mudstone. The image was acquired by MAHLI on sol 169, ID 0169MH0002080020102218C00. (F) John Klein drill hole (see Fig. 3) reveals gray-colored cuttings, rock powder, and interior wall. Note the homogeneous, fine grain size of mudstone and the irregular network of sulfate-filled hairline fractures. An array of eight ChamCam shot points can be seen. The diameter of the hole is 1.6 cm. The image was acquired by MAHLI on sol 270, ID 0270MH0002540050102794C00.

sediments were derived from a provenance with a composition similar to average martian crust and that experienced negligible chemical weathering before deposition at Yellowknife Bay. Relatively subtle differences exist between the lower and upper parts of the Sheepbed, such that the top exhibits higher $\text{Al}_2\text{O}_3/\text{TiO}_2$ and Ni, consistent with a slight provenance change. A further change in trace elements (higher Cr, lower Ni and Ge), which occurs across the Sheepbed–Gillespie Lake contact, could result from either provenance differences or mineral sorting (e.g., chromite) associated with grain-size differences. Both APXS measurements and high-spatial resolution ChemCam targeted analyses provide evidence that crosscutting veins and fractures are composed of Ca-sulfate (see below).

The highest-resolution MAHLI images were acquired on sol 150 (Ekwir) and sol 169 (Wemecke) after brushing of the outcrop (Fig. 5E). These images reveal a very fine grain size based on the outlines of darker grains, and nearly all discernible grains are finer than 50 μm . By definition, a mudstone is a fine-grained sedimentary rock composed of 50% or more particles smaller than 62 μm (29). Although we cannot directly resolve and observe these <50- μm grains, we do know that ~20% are clays. It seems likely that the entire Sheepbed member is finer than most facies observed by Curiosity during the traverse down to Yellowknife Bay. Drilling of the outcrop produces gray powder and cuttings that are substantially different in color from the modern surface (Fig. 5F). Inspection of the drill hole reveals a gray rock that is fine-grained and homogeneous in texture, with the exception of light-toned fractures filled with minerals.

Independent evidence for the fine grain size of the Sheepbed mudstone comes from inferences of the physical responses of brushing and measurements made during drilling. The rotary motion of bristles during brushing substantially scored the surface of the outcrop in a fashion that shows deflections (Fig. 5E) around apparently harder, embedded dark nodules, suggesting that the mudstone matrix is softer. Comparison with terrestrial analog data suggests weak to medium hardness for the mudstone (30). Additional estimates of rock hardness are provided by comparison of rover engineering testbed data derived from terrestrial analogs with drill hardness data from the Sheepbed member (31). These data sets suggest that the Sheepbed rock is comparable to fluvial-to-lacustrine siltstones and mudstones collected from the ~10-million-year-old Ridge Basin of southern California (buried to depths of several kilometers) but not as hard as feldspathic sandstones derived from the same basin or as soft as pure commercially available kaolinite.

Sheepbed mudstones contain a few very thin intercalated beds that resist erosion (Fig. 4). These interbeds have not been studied in detail, but Mastcam data show them to be 1 to 2 cm thick and with substantial lateral continuity, as some can be traced for more than 100 m. Over-

all, the primary texture and grain size has a very uniform, massive appearance; it is well known, however, that lamination can be difficult to detect in many mudstones (29).

The very fine grain size, its apparently uniform distribution through the member, and the substantial lateral extent of centimeter-scale interbeds are all consistent with deposition of the primary grains due to settling from suspension, either from the atmosphere or a water column. The former might involve volcanic ash, eolian dust, or very distal impact fallout. Because the composition of the Sheepbed mudstone is basaltic and we cannot ascertain the very finest grain sizes, it is hard to discount an airfall origin. However, mapping relations, regional context, and local sedimentologic constraints all point to a distal alluvial fan or proximal lacustrine setting. In this setting, sediment-laden fluvial flows can deposit extensive fine-grained deposits, either as overbank flood deposits or at their downstream termination where they enter a body of standing water, such as a lake. Here, the decrease in flow turbulence as flows thin overbank and spread when leaving a channel, or encounter the still water of a lake, leads to deposition of suspended fines. We favor the lake interpretation because of the lateral extent and thickness of the Sheepbed member, the absence of fluvial channel bodies laterally adjacent to mudstone beds, and the fact that the mudstone underlies the Gillespie Lake member fluvial sheet sandstones over a large areal extent. Given our limited observations, it is difficult to exclude the hypothesis that this lake may have been seasonally dry (playa), or whether it responded to longer-term wet-dry climatic fluctuations. However, the compositional data favoring low salinity for the lake waters (19) and specific diagenetic textures discussed below (cement-filled syneresis cracks, rather than sediment desiccation cracks) suggest that it was perennially wet for the stratigraphic interval we examined.

Strata dominated by fluvial sediments that predate the advent of terrestrial vegetation on Earth are generally devoid of fines, except as occasional mud drapes that are preserved between sets of cross-stratified dune or bar deposits (32, 33). Sweeping of very broad and shallow channels that produce sheet flood deposits result in reworking of thin floodplain deposits that tend to not be preserved in the stratigraphic record. In addition, eolian reworking between flow events, in the absence of terrestrial vegetation to anchor fines, is also regarded as an efficient mechanism to sweep fines away from sites of deposition. Thus, a shallow lacustrine environment, which sequesters fine sediments over long time scales, is considered to be the most likely interpretation for the Sheepbed mudstone.

The grain size and composition may be inconsistent with typical Mars dust, which is even finer in grain size and characterized by SO_3 contents approaching 7% (20, 34), rather than

the Sheepbed mudstone, which is closer to 1% SO_3 (19). Although it seems conceptually possible to deposit relatively thick intervals of dust (35, 36), this might not be expected on an alluvial fan that is intermittently active enough to result in the substantial amounts of coarser-grained sediment accumulation seen in the Yellowknife Bay formation. Furthermore, estimates of dust accumulation rates are very low [on the order of 10 cm per million years (37)], as compared with fluvial or even lacustrine sediment accumulation rates, which are orders of magnitude higher (38).

Sheepbed compositions likely discount an origin as pure volcanic ash. Apart from variation imposed by sulfate-rich diagenetic features, the Sheepbed is geochemically uniform with an adjusted volatile-free composition close to average martian crust (19). Individual volcanic ash eruptions might be expected to display some variation in composition, and it would be coincidental for individual eruptions to all have a composition essentially the same as the average crust. If the >1.5-m-thick Sheepbed member represented a single eruption, this would require a local source that is not observed in the vicinity of Gale crater [probably within a few tens of kilometers for 1 m of thickness (39)].

Diagenesis of the Sheepbed Mudstone

A diverse set of diagenetic features that postdate deposition is observed in the Sheepbed mudstone member. Early diagenetic features include nodules, hollow nodules, and raised ridges (Figs. 6, A to C). These features are crosscut by later fractures, filled with light-toned cements (Fig. 6D) shown to be variably hydrated sulfate minerals based on ChemCam, APXS, Mastcam, and Chemistry and Mineralogy instrument (CheMin) data. Additionally, a dike-like feature known as the “snake” obliquely crosscuts the Sheepbed–Gillespie Lake contact and extends for several meters (Fig. 3).

Concentrations of nodules and hollow nodules have patchy distributions and pass laterally (Fig. 7) into mudstones that have raised ridges. Nodules are expressed as millimeter-scale protrusions of the outcrop with 3D differential relief suggesting crudely spherical geometry (Fig. 6A). The mean diameter of 4501 nodules is 1.2 mm, with minimum and maximum diameters of 0.4 and 8.2 mm, respectively. Brushing shows that nodules are more resistant to brush-induced etching and, thus, harder than the surrounding mudstone (Fig. 5E). Mastcam, MAHLI, and ChemCam Remote Micro-Imager (RMI) data show that nodules are dispersed from one another, cluster in patches (Fig. 7), do not form their own beds, and show no evidence of size grading, despite a broad range in diameters. These constraints suggest that the nodules are of concretionary origin rather than volcanic or impact lapilli (40, 41). Furthermore, no associated out-sized clasts that might be interpreted as potential bombs, or any breccias, are observed. Comparison of John Klein (fewer nodules and hollow

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nodules) and Cumberland (more nodules and hollow nodules) compositional data indicates that nodule and hollow-nodule rim growth may have involved iron-bearing compounds such as the mineral akaganeite or even clay minerals (19, 28).

Hollow nodules are millimeter-scale circular rims with hollow centers (Fig. 6B). Three-dimensional exposures sometimes reveal pedestal-like structures with a circular rim surrounding a hollow, bowl-like depression. The outcrop therefore has a locally pitted appearance, suggesting erosional exposure of approximately spherical voids that had resistant rims, similar to the nodules. Resistance to weathering by the rims of the hollow nodules is indicated by the presence of lineations and “wind tails” carved into the outcrop, presumably by eolian saltation abrasion. Development of pedestals likely records lowering of the outcrop surface by prolonged eolian abrasion. Mastcam, MAHLI, and ChemCam RMI data show that hollow nod-

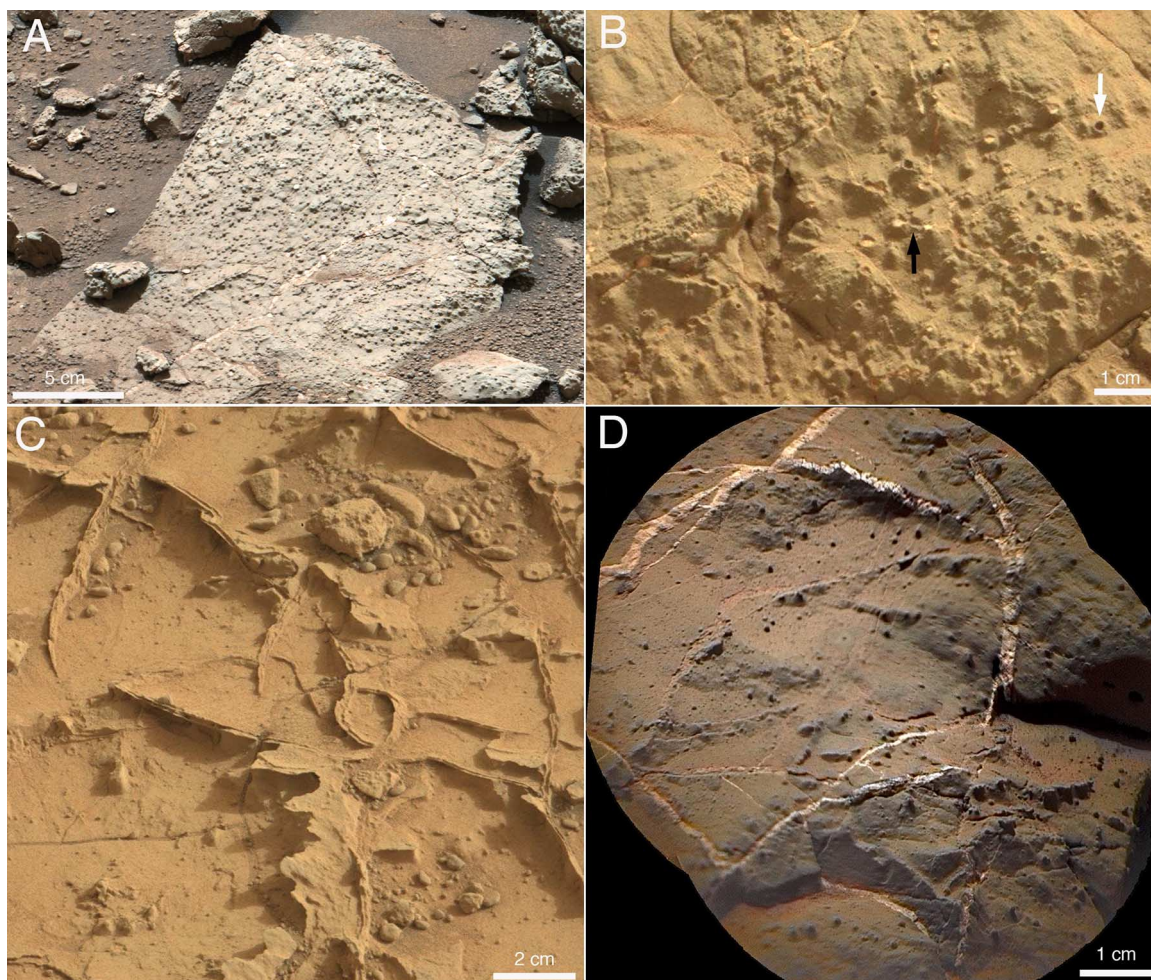
ules are similar in size to nodules with mean diameters of 1.2 mm, as well as minimum and maximum diameters of 0.6 and 5.6 mm, respectively ($n = 1248$ hollow nodules). We note that the upper size limit of hollow nodules is considerably smaller than that for nodules. The interior mean diameter of the void space defining the hollow is 0.7 mm ($n = 45$).

Hollow nodules are regarded as void spaces preserved within the Sheepbed mudstone. However, the hollow nodules are distinctly different from millimeter-scale diagenetic vugs, such as in the Gillespie Lake member or Burns formation of Meridiani Planum (40), in that the hollow nodules are associated with a ubiquitous circumscribed rim. Spherical voids of this type have not been observed previously on Mars and perhaps relate to their ability to be preserved—in this case, within fine mudstone sediments. Two options are possible to explain the void space,

which creates the hollow observed in outcrop: In the first hypothesis, the void space would represent a vanished mineral, whose higher solubility allowed easy dissolution during subsequent diagenetic fluid percolation events. Such minerals might have grown displacively in the sediment [for example, in evaporites (26)], thereby eliminating the need for the void to have been primary. However, no examples of filled hollow nodules were observed, with one very specific exception: Where hairline fractures filled with light-toned sulfate minerals intersect hollow nodules, the hollow nodules also are filled with sulfates. Otherwise, hollow nodules are devoid of any remnant of an earlier mineral. The sulfate-filled fractures represent a later phase of diagenesis (see below), and therefore, the sulfate minerals are not considered part of the primary environment.

This allows for a second hypothesis in which the hollow nodules represent primary voids,

Fig. 6. Diagenetic features of the Sheepbed member. (A) The Sheepbed target shows that millimeter-scale nodules, interpreted as concretions, are locally abundant. Note their dispersed distribution. The outcrop is crosscut by sulfate-filled fractures. This image is a subset of a mosaic acquired by Mastcam34 on sol 192. Image IDs are 0192ML-0010170240105705E01_DXXX and 0192ML00101-70070105688E01_DXXX. The image has been white-balanced. (B) Hollow nodules at the Bonnett Plume target (see Fig. 3) are defined by circular cross-sections through voids with raised rims (white arrow). Voids are filled with sulfate minerals where intersected by hairline fractures (black arrow) but are otherwise unfilled. The image was white-balanced and acquired by Mastcam-100 on sol 159. Image ID is 0159MR0008640050-201360E01_DXXX. (C) Raised ridges near the McGrath target have spindle-shaped terminations (see Fig. 3). Note the broadly varying strikes with dips ranging from vertical to 45°. Ridges most commonly have parallel sides, but they also have one or two additional infilling bands. All contour the local topography of the fracture margin and are interpreted as infilling cements. The image was white-balanced and acquired by Mastcam-100 on sol 164. Image ID is 0164MR0008850000201589E01_DXXX. (D) Sulfate-filled fractures at the Sheepbed target (see Fig. 3) are consistent with brittle deformation of lithified mudstone, followed by precipitation of sulfate from



mineralizing fluids. Note the abundant millimeter-scale nodules. The fracture fills are mostly flush with the bedrock surface, which contrasts with the nodules that weather in relief. This image is a ChemCam RMI mosaic that has been pan-sharpened using white-balanced Mastcam-100 color data. Images used in this composite are CR0_408676341EDR_F0051398CCAM01126M1, CR0_408675268EDR_F0051398CCAM01126M1, and Mastcam-100 ID 0126MR0007800000200786E01_DXXX.

such as gas bubbles formed during or soon after deposition. Several mechanisms could generate gas during or after sedimentation, including diagenetic dissolution and precipitation reactions that might produce gas as a by-product. These might include hydrogen gas produced during authigenic clay mineral formation via olivine “saponitization” (28); ultraviolet (UV) photo-oxidation of reduced minerals, such as siderite, to produce authigenic iron oxide (e.g., magnetite) and hydrogen gas (42); pressure-induced release of dissolved gas from sedimentary pore fluids; and processes that reflect distinct Mars environments, such as boiling of water in the presence of critically low atmospheric water vapor pressure. Data are insufficient to distinguish among these possibilities. However, the last of these can be discounted by the presence of thin interbeds within the Sheepbed mudstone, which suggests that wholesale churning of the mudstone did not occur. A final possibility considers the potential role of microbes, if life had ever evolved

on Mars. Terrestrial sediments are pervaded by microbes, which produce a variety of gases that become trapped as bubbles where lithification is early and rapid (43). However, it should be clear enough that this mechanism can only be invoked as a serious possibility after all other abiotic hypotheses have been discounted. This is not the case for our current data set, and we include this only for the sake of completeness.

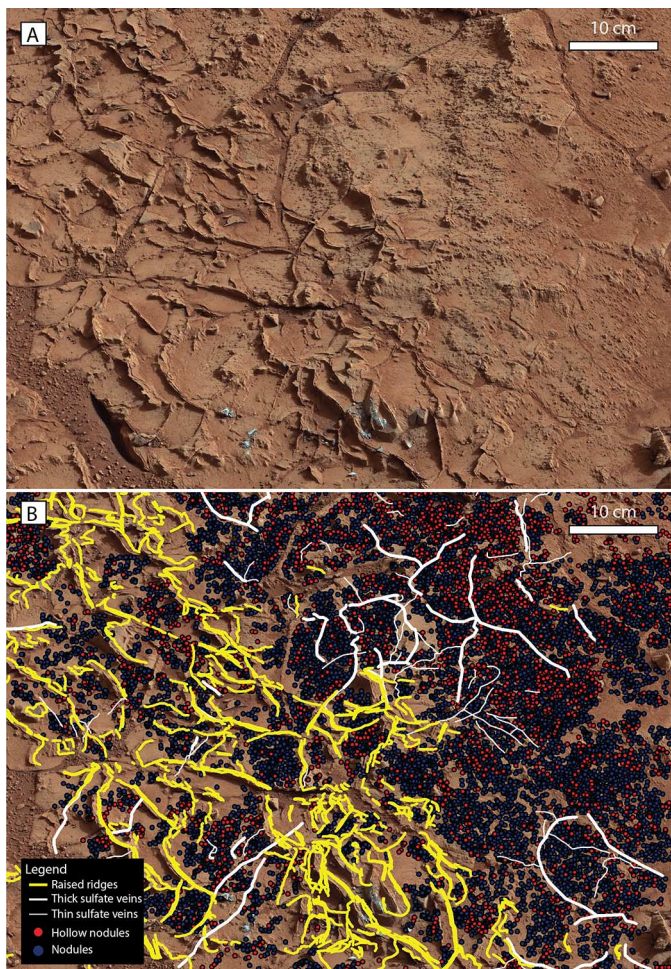
Raised ridges (44) are narrow, curvilinear ridges that weather in raised relief, and, as mentioned above, they substitute laterally for nodules and hollow nodules (Fig. 7). The raised ridges are restricted to the Sheepbed mudstone, but one poorly constrained example was noted at a higher stratigraphic level (observed only from a distance; exact stratigraphic position and facies are uncertain). Measurement of 1619 raised ridges in the Sheepbed suggests that they extend at least several centimeters in length and have a mean width of 2.7 mm, with maximum widths of up to 5.8 mm. Raised ridges have spindle-

shaped, pointed terminations (Fig. 6C). Most have subvertical orientation but can dip more gently, down to 45° or less, and their strike trends in all directions (Fig. 6C). Intersections of 90° between ridges are common. A distinctive attribute is that most ridges show internal banding, which contours the trend of the ridge, following closely all local deviations, including right-angle bends. Two parallel bands are most common, although three to four are also observed (Fig. 6C). Raised ridges are most simply interpreted as early diagenetic cracks that formed in consolidated or even partly lithified mudstone, then filled with one or more generations of cement that isopachously encrust the margins of the cracks. This infilling material has greater resistance to eolian erosion than the mudstone, resulting in its expression as ridges. ChemCam and APXS analyses have identified elevated Mg, Fe, Si, Cl, Li, and Br in the raised ridges, but insufficient evidence exists to confidently identify specific mineral phases (19). A strong correlation exists between Mg, Fe, and Cl, possibly indicating that a chloride phase may be present, but if so, it can only explain a small amount of the Mg and Fe enrichment. Alternatively, the correlation could result from a phase other than a chloride that contains substantial Cl. For example, CheMin data suggest the presence of akaganeite (28).

Taken alone, the development of very early cracks in fluvial-to-lacustrine mudstones might be most simply explained by desiccation of wet sediment: Generally, desiccation cracks have polygonal geometry. Therefore, the nonpolygonal geometry of the cracks is unexpected, as are the isopachous, banded materials that fill in the fractures. Furthermore, the abundance of raised ridges is anticorrelated with the abundance of nodules and hollow nodules, suggesting that all three phenomena are genetically related, which is not observed for desiccation cracks on Earth. An alternative mechanism proposes that syn-depositional pore fluids (perhaps mixing with surface runoff waters) may have been important in driving diagenetic reactions that triggered early mineral precipitation, gas production, and volume contraction close to the sediment-water interface. On Earth, a related but poorly understood process known as “synaeresis” is thought to relate to clay mineral flocculation during mixing of saline and less saline waters, resulting in differential contraction of the substrate (45). Seismic shaking, perhaps induced by impacts on Mars, may help facilitate this process (46). The result is spindle-shaped cracks, a few centimeters long, that look very much like the Sheepbed raised ridges. In most cases on Earth, the cracks are filled by other sediments; however, in one very particular instance known as “molar tooth structure,” spindle-shaped cracks and related subspherical voids are filled with mineral (calcite) cement. The origin of subaqueous cracks on Earth is poorly understood, though the involvement of gas in generating the cracks and voids

Fig. 7. Map of diagenetic features showing spatial relations between fabric elements. The rock surface coincides with a bedding plane. Note that nodules and hollow nodules pass laterally into raised ridges, suggesting variations in lithologic or diagenetic fluid properties (see text).

(A) A subset of the Mastcam-100 workspace mosaic acquired by the Curiosity on sol 164. This perspective mosaic is white-balanced and was used as a base map. (B) The sol 164 workspace mosaic annotated to show the distribution of early diagenetic features (nodules, hollow nodules, raised ridges) and later diagenetic features (thick to thin sulfate-filled fractures). Erosion-resistant raised ridges were outlined individually and generally exhibit parallel sets of isopachous linings. Thin (<5 mm in width) sulfate veins have relatively constant thickness, whereas thicker sulfate veins have more variable thickness. Both sets of veins crosscut other diagenetic features. Hollow nodules are circular features (~1 mm in diameter) that stand out in raised relief from the outcrop surface and are distinguished from nodules by the presence of a small depression in the center of the feature. Because hollow nodule diameters are often at or below the resolution limits of this mosaic (confirmed by MAHLI observations), hollow nodules are probably underrepresented relative to nodules. This image is a subset of the Mastcam-100 workspace mosaic from sol 164. For mosaic image IDs, see (31).



represented by molar tooth structure is likely (43, 47). As with the hollow nodules, a number of processes could generate gases in martian sediments. In general, the distribution of subaqueous shrinkage cracks reflects differential strength of the substrate materials, which may similarly be represented in the Sheepbed member by the spatial segregation of the nodules and hollow nodules versus raised ridges.

A distinct feature within lower Yellowknife Bay strata is a dike-like, dark-toned rock unit referred to as the “snake,” named after the target Snake River (see Fig. 3). This feature extends from the lowest stratigraphic level of the exposed Sheepbed member, crosscuts the Sheepbed–Gillespie Lake contact, and tapers out within the middle of the overlying Gillespie Lake member (Figs. 3 and 4). The snake is 6 to 10 cm wide and can be traced across bedding for ~5 m, cutting up through almost 1 m of stratigraphic section. MAHLI images of unbrushed parts of the snake suggest that it has a fine-grained clastic texture, but with larger fragments present suggestive of intraclasts (fig. S2). Elemental data show it to be similar in major elements but distinct from the Sheepbed member in trace element abundances (19). The snake can be traced to its stratigraphically highest position, where it terminates in a small anticline. At this terminus, thicker, massive beds extend laterally away from the core of the anticline. The snake has several possible interpretations, including a narrow igneous dike of basaltic composition; a large-scale crack that is filled from above, perhaps associated with subaerial exposure or ice wedging; or a sedimentary intrusion associated with compaction and dewatering of the mudstone or subjacent strata.

The fine-grained, basaltic composition of the snake makes it hard to exclude an igneous intrusion with basaltic composition. The feature is so thin that grain-size variations consistent with chilled margins would be unexpected at scales within MAHLI’s limit of resolution; the entire dike would be expected to be very fine grained. Filling from above is discounted due to the presence of the small anticline, which suggests injection of fluidized material from below rather than settling of sediment from above. In addition, no evidence of layering, suggestive of downward percolation of sediments, is observed within the feature. Furthermore, no other crack-like features are observed to extend downward from a common stratigraphic surface. The third possibility—intrusion of plastically deformed sedimentary materials—occurs in some sedimentary environments with high sedimentation rates (24, 25, 48, 49). Upward and lateral injection of compacting muds and sands is most commonly expressed as dikes and sills that both crosscut strata and intrude along bedding planes. The similarity of both composition and texture to that of the Sheepbed mudstone, the presence of possible intraclasts, and upward deflection of strata at its terminus are all consistent with

sedimentary injection (24, 25). This suggests a sediment accumulation rate for the Yellowknife Bay formation (or sediments below it) that was high enough so that pore fluid pressures built up to the point where expulsion along discrete zones of weakness, rather than along grain boundaries, was the preferred pathway for escape of compacting sediments and interstitial pore fluids. As the porosity and permeability of the sediment was reduced via compaction and cementation, the strata may have ruptured, allowing fluids to escape and form sedimentary dikes and sills. Finally, this process could have been aided by impact-induced seismic activity (50).

Fractures filled with light-toned sulfate cements also are regarded to have formed later in the diagenetic sequence because they cut the entire Yellowknife Bay formation. In contrast to the raised ridges, the sulfate-filled fractures are flush, even slightly depressed, with respect to adjacent unfractured outcrop (Figs. 6 and 7). Sulfate-filled fractures have highly diverse orientations spanning from vertical to subhorizontal; range in length from less than 1 cm to more than 30 cm; and range in width from “hairline” (submillimeter) to 8 mm, with a mean width of 2.3 mm (Figs. 5E and 6D). MAHLI images of the John Klein drill hole show that sulfate-filled fractures of hairline width have complex orientations with horizontal as well as vertical trends (Fig. 5F). Where these hairline-width fractures intersect hollow nodule void spaces (Fig. 5E), the latter are also filled with hydrated and anhydrous calcium sulfate minerals, as determined by CheMin, ChemCam, and APXS data (19, 28). At a larger scale, the observation of fracture-filling hydrated sulfate mineralogy can be extended through Mastcam observations. Calibrated Mastcam spectra show evidence for hydration associated with most but not all of the fracture fills, including limited hydration in hairline fractures that penetrate and fill the void space of hollow nodules (28).

To form, the sulfate-filled fractures first require lithification of the Sheepbed and Gillespie Lake members to allow applied stress to cause brittle strain of the bedrock (51). Once open, the fracture networks then act as conduits for fluid transport, resulting in precipitation of cementing minerals within these initially low permeability sedimentary rocks. The fracture networks were possibly created by hydraulic fracturing due to high fluid pressures that would have been obtained during burial of Yellowknife Bay strata (51). The variation in hydration state of vein-filling sulfate mineralogy between gypsum, bassinite, and anhydrite could reflect primary variations in environmental conditions during deposition or, alternatively, diagenesis associated with burial and/or desiccation during subsequent erosion and exposure (28).

A final observation regarding later diagenesis concerns the Gillespie Lake sandstone member, which shows evidence for possible secondary porosity. Irregular vugs larger than the diameter of the average grain size (Fig. 5D) suggest that

larger grains, or perhaps clusters of grains, were leached during fluid circulation. Alternatively, it is possible that these vugs represent intraclasts of mudstone that degraded by weathering. Another possibility is dissolution of preferentially more soluble early diagenetic minerals; however, the irregular shape of the vugs is not consistent with dissolved evaporite crystals.

The diagenesis of the Sheepbed member suggests a complex aqueous history involving at least two distinctly different water masses. After emplacement in distal alluvial-to-lacustrine environments, pore spaces in primary sediments were occluded by cements, and early diagenetic hollow nodule voids (gas bubbles?) may have been formed, along with concretions. At approximately the same time, nonpolygonal fissures in the lithifying sediment opened up and became lined with void-filling isopachous rim cements. All of these events, with the exception of hollow nodule void formation, must have occluded much of the primary porosity. This episode of diagenesis did not substantially change the bulk rock chemistry of these units relative to average martian basalt and are thus considered to have involved isochemical alteration (19). This suggests that clay mineral and magnetite formation happened largely in situ, as a result of alteration of olivine to form authigenic saponitic smectite (19, 28). In addition to pore-filling cement, it is possible that these minerals also precipitated as concretions, as well as isopachous rim cements within raised ridge fractures. After lithification, fracture porosity was created that was then filled with a distinctly different fluid that resulted in precipitation of sulfate cements (19, 28). The absence of elevated sulfate abundances in the Sheepbed mudstone, except for within these fractures, indicates that cementation and permeability reduction of the mudstone was extensive enough so that sulfate-rich fluids percolating through fractures and hairline fractures did not invade the mudstone or sandstone matrix (19, 28). The first diagenetic fluid, possibly derived from the waters that transported sediments, was dominated by dissolved silicate and iron oxide minerals. The second, subsequent fluid was dominated by sulfates and perhaps sampled a broader, basin-wide hydrologic system. CheMin and Sample Analysis at Mars instrument (SAM) data additionally point to a substantial x-ray amorphous fraction of material that could have been involved in diagenesis, as well as potential sulfide minerals (28, 52).

Sheepbed Mudstone: Record of Habitable Environment

The regional geologic context, sedimentologic framework, and geochemistry and mineralogy of the Sheepbed mudstone all point to an ancient environment that would have been habitable to a broad range of prokaryotic microorganisms. The relatively neutral pH indicated by clay mineral stability (28); high water activity indicated by low salinity for primary and early

diagenetic environments (19); the variable redox states indicated by presence of magnetite, sulfate, and sulfide minerals and compounds (28, 52); and the apparent long duration of the aqueous environment(s) all support this interpretation.

The Sheepbed mudstone closely coincides with the BF map unit (Fig. 1), suggesting that this rock unit may have extended beyond the confines of Yellowknife Bay and minimally covered an area of ~30 km², not including buried or eroded equivalents. Repeated flows of water (17) and the likely presence of a body of standing water point to a sustained habitable environment. The durations of the primary, early diagenetic, and later diagenetic aqueous environments were substantial enough to create geologic and geochemical fingerprints of prolonged fluvio-lacustrine and diagenetic processes markedly similar to those that formed commonly during early Earth history (32, 53, 54).

A rough estimate of the minimum duration of the lacustrine environment is provided by the minimum thickness of the Sheepbed member. Given 1.5 m, applying a mean sediment accumulation rate for lacustrine strata of 1 m per 1000 years (38) yields a duration of 1500 years. Lacustrine sediment accumulation rates vary by several orders of magnitude (38); thus, the hypothesized lake could represent hundreds to tens of thousands of years. If the aqueous environments represented by overlying strata are considered, such as the Gillespie Lake and Glenelg members, then this duration increases. Indeed, the regional geologic context suggests that the Yellowknife Bay formation may be just a small part of a thick series of fluvio-lacustrine rocks that may exist in the subsurface for hundreds of meters. Moreover, it is possible that hundreds of meters of fluvial-lacustrine strata have been eroded. The sulfate-filled fractures are consistent with some amount of burial, and independent support for this is allowed (but not required) by possible incipient chloritization of mudstone smectite (28). Therefore, it is entirely plausible that, collectively, these hundreds of meters of strata could represent millions to tens of millions of years.

Episodic drying of this lake environment seems certain over this long time scale if not over shorter spans represented by thicknesses equivalent to the exposed Sheepbed mudstone. However, even if the lake was dry periodically or if unconformities interrupted the continuity of fluvial-lacustrine sedimentation, the presence of former groundwater—well documented for many ancient Mars phenomena (55–57)—may have provided a refugium to sustain a chemolithotrophic biosphere. Episodic lake rejuvenation would permit a subsurface chemoautotrophic microbiota to emerge to the surface in response to a rise in groundwater level.

We can more specifically assess the habitability of the former environment represented by the Sheepbed mudstone from a perspective shaped by our knowledge of terrestrial biology. This ap-

proach involves identification of the chemical and physical environmental requirements that would support prokaryotic microbes, if similarly simple forms of life had ever evolved on Mars. Bacteria and archaea represent ancient, abundant, and metabolically diverse domains of life on Earth and are able to withstand a wide range of environmental conditions (58–61). They can engage in a multitude of autotrophic and heterotrophic pathways and use a variety of electron donor-acceptor pairs for acquisition of carbon and energy (62–65). We can envisage many combinations of terrestrial microbes that would be suited to form a martian biosphere founded on chemolithoautotrophy at Yellowknife Bay.

As a first step, it is useful to assess the key nutrients required to support terrestrial bacteria and archaea (66). The defining chemistry is inorganic: elemental inventory, liquid water, redox potential (Eh), pH, redox couples, and a diversity of chemical compounds. Organic molecules are not required environmental constituents because they can be synthesized *de novo* through the primitive autotrophic pathways, such as methanogenesis and acetogenesis (67). We have measured H, O, S, C, N, and P in minerals and other compounds with the APXS, ChemCam, CheMin, and SAM instruments, as well as important metals such as Fe, Mg, and Mn (19, 28, 52). All but P are observed in SAM evolved gas analysis (EGA) data as volatiles, and all but P and N are known to be part of mineral structures observed in CheMin data. We do not have the capability to determine whether P would have been bioavailable, and CheMin observed no highly soluble P-bearing minerals. On the other hand, CheMin data indicate substantial breakdown of olivine in the mudstone at Cumberland (28), and P can be a trace-to-minor element in that mineral (68), suggesting potential P mobility during diagenesis. Nitrogen-bearing compounds, in both reduced and oxidized forms, were measured by SAM during EGA (52). Evolved NO (mass-to-charge ratio = 30) has an abundance that is substantially above background, pointing to a likely source within the mudstone. Bioavailable N could conceivably have been derived from the atmosphere via fixation by sulfide minerals during reactions similar to that seen in pyrrhotite, pyrite, and magnetite assemblages on Earth (69, 70).

SAM EGA data from John Klein show carbon to be present, either in crystalline (carbonate?) phases below 1% abundance, as a carbonate-bearing component in the x-ray amorphous material detected by CheMin, or derived from complex organic sources not detectable by SAM. Thus, most or all the major ingredients required to support life are present in the Sheepbed mudstone.

As a second step, we identify plausible redox couples represented by minerals and other compounds preserved in the Sheepbed mudstone that might have comprised basic energy-yielding reactions for an elementary microbial community (66). The presence of both sulfur and iron is indicated by ChemCam, APXS, CheMin,

and SAM data. Further, observation of key inorganic volatiles such as H₂O, H₂S, SO₂, H₂, and CO₂, generated during SAM pyrolysis (52), suggests a wider range of redox conditions for the surface of ancient Mars than previously recognized based on Viking, Phoenix, and Mars Exploration Rover data (71–74). For example, SAM analysis of the John Klein sample shows higher H₂S/SO₂ average ratios than were found for the Rocknest soil sample: 0.06 for John Klein (52) versus 0.01 for Rocknest (75). These data suggest a higher proportion of reduced sulfur relative to oxidized sulfur pointing to a plausible redox couple for prokaryotic respiration.

Iron also presents intriguing possibilities for redox coupling. CheMin analyses indicate the presence of a substantial quantity of likely authigenic magnetite (~7% of crystalline components) in Sheepbed mudstones (28). The presence of an authigenic, partially reduced Fe-oxide phase in a martian sedimentary rock represents a notable departure from previous secondary Fe-mineral detections on Mars, where ferric iron has proven to be the dominant redox state (76–78). The magnetite measured by CheMin does show evidence for oxidation of some of its ferrous iron to form the ferric defect-spinel maghemite (28). The gray color of the freshly exposed Sheepbed mudstone (Fig. 5F) contrasts sharply with the reddish color of the freshly exposed, highly oxidized, hematite-bearing rocks of Meridiani Planum (79), emphasizing the lower oxidation state of iron in the Sheepbed mudstone. There are a number of potential mechanisms for the production of authigenic magnetite, all of which are promoted by waters with relatively low Eh and neutral to alkaline pH (80). Under such conditions, aqueous Fe²⁺-hydroxide species can precipitate and ultimately transform to magnetite. Perhaps not coincidentally, neutral-to-alkaline pH conditions also are likely to promote the formation of clay minerals. Potential authigenic magnetite formation pathways include UV-promoted reactions with dissolved Fe²⁺ in the water column (81) or with carbonate minerals in the sediment (42), and partial oxidation of dissolved Fe²⁺ by dissolved O₂ (80). Perhaps the most likely scenario is via clay formation and saponitization (28). However the magnetite may have formed, the additional presence of Fe³⁺ in nanophase iron oxide indicated by combined APXS/CheMin and SAM data (19, 28, 52) provides the other half of an iron-based redox couple in the Sheepbed mudstone.

Salinity provides another important constraint on habitability because it affects water activity, which, when low, can restrict cellular physiology (82–84). All natural waters contain dissolved ions that, through their interactions with water molecules, limit the availability of H₂O for hydration reactions. Water activity is defined by microbiologists as $a_w = n_1/(n_1 + n_2)$, where n_1 equals moles of water and n_2 equals moles of solute (82). Most terrestrial organisms cannot survive below $a_w = 0.9$, a few halophilic

bacteria grow at $a_w = 0.85$, and some archaea can grow at $a_w = 0.75$ (83). In the Burns formation at Meridiani Planum, the abundance of calcium and magnesium sulfate minerals detected by the Opportunity rover is so great that it conservatively reconstructs to an ancient water activity of $a_w = 0.78$ to 0.86, and possibly as low as $a_w = 0.5$ (85). This shows that water activity was so low during deposition and diagenesis of the Burns formation that even though water was present, the environment would probably have been uninhabitable to all but the hardest halophiles (86). In contrast, the Sheepbed mudstone provides evidence for both low salinity sediment-transporting water and early diagenetic water, given its very low sulfur and chlorine content (19). It is possible that small amounts of chloride salt were present (19, 28); however, these were in low enough abundance (<1 to 2%) so as to not substantially reduce water activity and create a challenge for prokaryotic microbes. Higher salinities are indicated for the waters that percolated through late diagenetic fractures; however, these were probably of modest salinity given their exclusively calcium sulfate composition (85). On Earth, calcium sulfate precipitation is common in evaporitic environments that thrive with diverse microorganisms (87). Furthermore, there is evidence that halophiles may resist the destructive effects of radiation (88), thought to limit habitability in the martian surface environment (89).

Finally, estimates of pH are similarly important for understanding habitability. Again, in the Burns formation at Meridiani Planum, the presence of jarosite indicates low pH with ancient waters that contained sulfuric acid (40). However, such low pH does not discount the presence of life; terrestrial analog environments show that microbes can survive under these conditions because they have adapted their biochemistries to maintain near neutral conditions internally (90). Nevertheless, the presence of more neutral waters allows a broader range of microorganisms to be viable. The presence of clay minerals in the Sheepbed mudstone, the absence of any evidence for Al mobility, and the absence of iron sulfate minerals, even in the sulfate-filled late diagenetic veins, indicates a relatively neutral pH. This neutral pH could have existed for all phases of aqueous history, from the primary waters of deposition that may have created a body of standing water, to early diagenesis, and through the close of late diagenesis.

At Yellowknife Bay, there is no hint of the strongly acidic conditions that have been thought to especially describe the planet's younger history of aqueous alteration, sedimentation, and habitability (74, 83, 91). The record of aqueous activity at Yellowknife Bay is likely prolonged and complex, involving several stages of diagenesis, including clay formation, that culminate with precipitation of calcium sulfate salts in veins, but without attendant indicators of acidic waters such as iron sulfates. The sim-

plest interpretation of the sequence of diagenetic events would involve progressive desiccation of mildly saline, pH-neutral waters—a very Earth-like scenario (92). Such conditions have been envisaged for the very early history of Mars (91), but it is only recently that they have been considered viable for a younger age (7, 93, 94). The surprising result is that the stratigraphy of Yellowknife Bay may not only preserve evidence of a habitable environment, but one that is relatively young by martian standards. In the most conservative scenario allowed by geologic mapping, the Yellowknife Bay formation represents part of the older fill of Gale crater, and therefore is roughly Early Hesperian in age (6), perhaps overlapping with or postdating times of bedded sulfate formation elsewhere on Mars. This would indicate that times of sustained surface water, neutral pH, and authigenic clay formation extended later into Mars' history. The potentially young age of clay formation (and habitability) does not invalidate the general trend that most rocks that interacted with water early in Mars' history produced clays, and those that interacted later produced sulfates. However, much like Earth's time-dependent records of iron formation (95, 96) and carbonates (97, 98), it points to the need to understand those special conditions, which allow a distinctive aqueous environment to persist for broad spans of geologic time or to recur when the favorable conditions again emerge. Curiosity's detection of a relatively young and markedly Earth-like habitable environment at Gale crater underscores the biologic potential of relatively young fluvio-lacustrine environments.

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10. This decision was made by the entire MSL Science Team with strong consensus to drive a relatively short distance (~500 m) to examine rocks with higher thermal inertia values that also had an apparently strong spatial association with the AF unit. The prediction based on mapping was that fluvial and/or lacustrine rocks potentially derived from the crater rim might be encountered. The strata exposed at the base of Mt. Sharp are still considered to be the primary mission objective, and the team has established a departure date from Yellowknife Bay of the first week in July 2013.
11. The names used for rock and soil targets studied by Curiosity and reported in this paper are derived from established rock formations of northern Canada. In turn, these names derive from names of local geographic features where they occur, per convention, such as mountains, streams, lakes, bays, etc. This choice of naming scheme celebrates the city of Yellowknife, Northwest Territories, long known to be a port of departure for geologic mapping expeditions into some of the oldest rocks of North America. Yellowknife crater was designated by the International Astronomical Union, and Yellowknife Bay demarcates the area into which Curiosity descended to study its geologic history.
12. A base map was created based on 25-cm-per-pixel HiRISE visible image data, 1-m-per-pixel elevation data, and 100-m-per-pixel Thermal Emission Imaging System data that was subdivided into 140 1.2° by 1.2-km (0.025°) quadrangles and mapped in detail by more than 30 team members. The group map was compiled, consolidated, and iteratively refined into a single GIS project.
13. In addition, minor units of eolian fill and/or bedforms and ejecta blankets adjacent to larger craters are present but are not discussed further.
14. Bradbury Landing refers to the patch of ground where Curiosity touched down on the surface of Mars. It commemorates the noted science fiction writer Raymond Bradbury and was designated by NASA.
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18. The morphology of the “bay” is the result of postdepositional erosion, perhaps by eolian processes, as shown by the exposures of stratified rock that record dissection. These are eroded rocks and not, for example, shoreline berms. Therefore, the present-day topography does not reflect that at the time of deposition of those strata.
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Acknowledgments: We are indebted to the MSL Project engineering and management teams for their exceptionally skilled and diligent efforts in making the mission as effective as possible and enhancing science operations. We are also grateful to all those MSL Science Team members who participated in tactical and strategic operations. Without the support of both the engineering and science teams, the data presented here could not have been collected. Some of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with NASA. Data presented in this paper are archived in the Planetary Data System (pds.nasa.gov).

Supplementary Materials
www.sciencemag.org/content/343/6169/1242777/suppl/DC1
 Supplementary Text
 Figs. S1 to S4
 Tables S1 and S2
 References (99, 100)
 MSL Science Team Author List

4 July 2013; accepted 6 November 2013
 Published online 9 December 2013;
[10.1126/science.1242777](https://doi.org/10.1126/science.1242777)



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Science **343**, (2014);

DOI: 10.1126/science.1243480

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Mineralogy of a Mudstone at Yellowknife Bay, Gale Crater, Mars

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Sedimentary rocks at Yellowknife Bay (Gale crater) on Mars include mudstone sampled by the Curiosity rover. The samples, John Klein and Cumberland, contain detrital basaltic minerals, calcium sulfates, iron oxide or hydroxides, iron sulfides, amorphous material, and trioctahedral smectites. The John Klein smectite has basal spacing of ~10 angstroms, indicating little interlayer hydration. The Cumberland smectite has basal spacing at both ~13.2 and ~10 angstroms. The larger spacing suggests a partially chloritized interlayer or interlayer magnesium or calcium facilitating H₂O retention. Basaltic minerals in the mudstone are similar to those in nearby eolian deposits. However, the mudstone has far less Fe-forsterite, possibly lost with formation of smectite plus magnetite. Late Noachian/Early Hesperian or younger age indicates that clay mineral formation on Mars extended beyond Noachian time.

The recent decade of orbiter- and rover-based studies of ancient sedimentary rocks on Mars has revealed a diverse mineralogy that constrains the nature and timing of early environments in the history of the planet (1–3). These studies provide a starting point for considering the habitability of Mars, based on an understanding of the aqueous geochemistry and mineralogy of rocks placed within a geologic framework (4, 5). Such an approach has been adopted by the Mars Science Laboratory (MSL) mission, where the science payload and advanced

capabilities of the Curiosity rover were designed for assessment of past habitability (6).

Mission goals for MSL placed high priority on aqueous-system mineralogy, particularly clay minerals and sulfate salts (6). The mission concept for the landing site in Gale crater was to leave the landing spot quickly and drive to Aeolis Mons, a central mound informally known as Mount Sharp. Interpretation of Mars Reconnaissance Orbiter CRISM (Compact Reconnaissance Imaging Spectrometer for Mars) visible–near-infrared (IR) spectroscopy suggests the presence of hydrated minerals in sedimentary layers at the base of the mound (7). However, soon after landing, a contact among three different geologic units, one with relatively high thermal inertia, was recognized within ~450 m of the landing spot, just beyond the alluvial lobe of the Peace Vallis fan (8). The decision to drive away from Mount Sharp toward this location has provided early samples of a mudstone that contains both clay minerals and sulfate salts.

The John Klein and Cumberland drill samples were collected from the Sheepbed mudstone member of the sedimentary Yellowknife Bay formation, which is interpreted as a shallow lacustrine deposit (8). John Klein and Cumberland are the second and third solid samples, respectively, collected by the MSL rover Curiosity. The first sample, from an eolian deposit named Rocknest, is ~60 m west of the mudstone drill locations. The loose Rocknest deposit (9) had been used to commission Curiosity's scoop sampling system, and the lithified John Klein sample was used to commission the drill. Curiosity's sampling system delivers both scooped and drilled powders to the same set of sieves (10). All scooped or

drilled samples were sieved to <150 µm, and portions were analyzed by the Chemistry and Mineralogy (CheMin) x-ray diffraction (XRD) and x-ray fluorescence (XRF) instrument (11) and the Sample Analysis at Mars (SAM) quadrupole mass spectrometer–gas chromatograph–tunable laser spectrometer suite of instruments (12, 13).

CheMin XRD data are the focus of this paper. Although the CheMin XRD instrument is the prime mineralogy tool carried by Curiosity, constraints on the mudstone mineralogy are provided by the temperatures at which volatiles are released in SAM evolved gas analyses, particularly H₂O release profiles (13). Other instruments on Curiosity provide additional insight into mineralogy. Mastcam (14, 15) multispectral images are capable of sand size resolution and potential identification of certain hydrated minerals using short-wavelength near-IR filters (16). ChemCam has a narrow laser beam that can target veins and nodules for remote chemical analysis by laser-induced breakdown spectroscopy (LIBS), with sensitivity to many elements including hydrogen (17, 18); this capability can aid in constraining mineral compositions where individual minerals are ~0.5 mm or larger and is particularly sensitive to alkali and alkaline-earth elements (19). The MSL alpha particle x-ray spectrometer (APXS) has proven heritage from previous missions and provides sensitivity to a wide range of common rock-forming elements (20), although the analysis spot resolution is ~1.7 cm, providing a bulk chemical analysis rather than mineral analysis for most samples. We use the results from APXS to constrain the composition of the x-ray amorphous components of the mudstone. The Mars Hand Lens Imager (MAHLI) provides high-resolution images, to 14 µm per pixel, with Bayer pattern color that can simulate hand-lens or close-up sample analysis (21).

Figure 1 shows Mastcam and MAHLI images of the boreholes and drill powders for John Klein and Cumberland. Cumberland (Fig. 1C) was targeted after John Klein so as to analyze a part of the mudstone with fewer sulfate veins and a greater abundance of resistant concretions, including nodules and hollow nodules that are regarded as early cementation. The two drill holes are ~3 m apart. Powders of both mudstone samples are notably gray in color, unlike the reddish weathering and/or dust evident on the surface of the mudstone. This reddish surface of the mudstone did not contribute to either drill sample, for the auger does not pass powder into the sampling system until it is ~1.5 cm into the drill target (10).

Mineralogical Analysis and Quantitative Mineralogy

The XRD patterns for John Klein and Cumberland are compared in Fig. 2A. We quantified the crystalline components, other than smectites, in John Klein and Cumberland (Table 1) by using whole-pattern fitting and Rietveld analysis (Fig. 2, B

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and C); smectites and the amorphous component were quantified using a modified version of the FULLPAT program (22). These methods are described in (23) and elaborated in (24). Conversion of the two-dimensional images of Debye diffraction rings on the charge-coupled device (CCD) to one-dimensional diffraction patterns was done in the same manner for Rocknest, John Klein, and Cumberland. For all three samples, we obtained unit cell parameters (Table 2) and phase compositions (Table 3) for major phases. The unit cell parameter and phase composition data reported for Rocknest, John Klein, and Cumberland were processed in the same manner and are therefore comparable.

An independent assessment of the abundance of smectite and amorphous material can be obtained from the fixed or cell parameter-constrained chemical compositions of the phases listed in Table 1 and the total sample chemical composition from APXS analysis of the drill tailings. This approach (24) uses a mass-balance calculation with XRD constraints on crystal chemistry (25, 26) and, for the present study, model smectite compositions, to estimate the composition of the amorphous component. Additional constraints on smectite abundance come from SAM evolved gas analysis, where the amount of H₂O released at higher temperatures (~400° to 835°C) can be related to dehydroxylation of various clay minerals (13). Within estimated errors these three methods agree, but in this paper we focus on the XRD estimates (Table 1).

None of the coarse (width >1 mm) veins that cross the Sheepbed member (8) were sampled for CheMin and SAM analysis. However, Mastcam near-IR spectral filters are sensitive to hydration in certain minerals (27), and this method (24) was used to analyze veins where ChemCam and APXS data indicated that Ca sulfate phases are present. This allows mapping of inferred gypsum distributions in the veins that were not sampled for CheMin and SAM analysis.

The sedimentologic context of the mudstone is complex. Observations of the mudstone (8) lead to interpretation of an early-diagenetic association of nodules, hollow nodules, and raised ridges that are crosscut by late-diagenetic fractures filled with light-toned Ca sulfates (S-Ca association in these veins was identified with ChemCam). Early-diagenetic hollow nodules are filled with light-toned sulfates only where intersected by late-diagenetic light-toned microfractures (8, 19). MAHLI images show that the John Klein drill spot had a surface footprint (1.6 cm diameter) with ~3.9% hollow nodules, ~2.5% solid nodules, and ~14.5% light-toned sulfate, whereas the Cumberland drill spot had ~8.5% hollow nodules, ~2.2% solid nodules, and no evident light-toned sulfate. Estimates of light-toned sulfate abundances from pre-drilling images can be deceptive because their three-dimensional distribution is dependent on variable occurrence of thin veins that may or may not be visible on the dust-mantled surface. A more accurate survey of the distribution and

abundance of light-toned sulfates is obtained by analysis of drill-hole wall images (24), at least to the depth exposed (the lower part of each drill hole contains some debris).

The borehole samples John Klein and Cumberland provide adequate sampling of the mudstone matrix with a partial sampling of features that are both early-diagenetic (nodules and hollow nodules) and late-diagenetic (light-toned veins). The drill did not sample any early-diagenetic raised ridges. The raised ridges were identified in LIBS and APXS analyses as including an Mg-Fe-Cl-rich component; in images they appear to have an isopachous filling of several layers and may be mineralogically complex (19). Without direct sampling and CheMin XRD analysis, knowledge of mineralogy in the raised ridges is speculative and is not addressed here.

Silicates Other than Smectites

Several detrital silicate minerals in the mudstone bear a strong resemblance to those found in the Rocknest eolian deposit (Tables 2 and 3). Fe-

forsterite, plagioclase, pigeonite, and augite are generally similar among Rocknest, John Klein, and Cumberland samples, suggesting similar mafic sources. Presence of pigeonite indicates mafic sources that were basaltic. However, XRD analyses of the mudstone samples reveal presence of orthopyroxene as well as clinopyroxenes, indicating a source of some mafic minerals that is either absent from or very minor in the nearby eolian deposit.

It is notable that Fe-forsterite is almost absent in Cumberland and that its abundance in John Klein is much lower than in Rocknest. Figure 1B shows that the reddish Rocknest sample coats the walls of the scoop that is filled with the John Klein drill powder. Testbed operations on Earth suggest that at least ~4% cross-contamination should be expected between samples. By the time Cumberland was imaged (Fig. 1D), the red Rocknest powder was almost gone, so the sampling system had been largely cleared of this contaminant in processing John Klein. Progressive dilution and a stronger cleaning cycle between

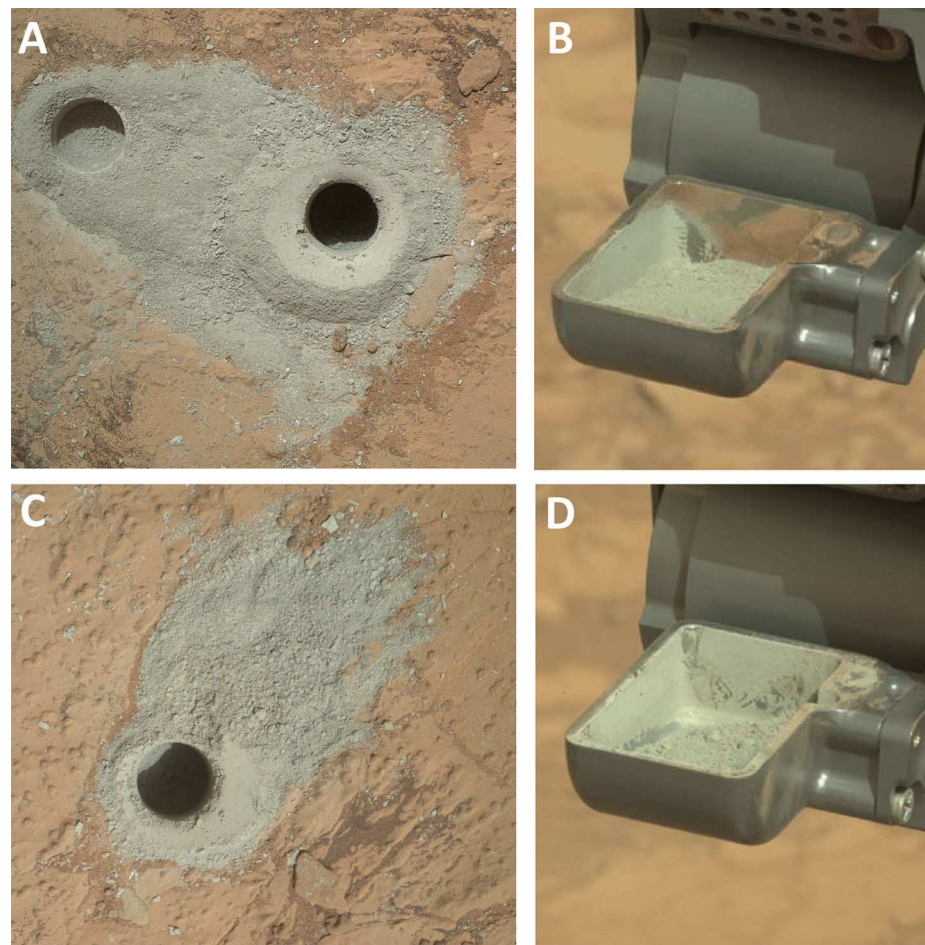


Fig. 1. Images of mudstone drill holes and drill powders. (A) shallow (1 cm deep) test drill hole (at left) and sampled drill hole (6.5 cm deep) for John Klein; MAHLI image, sol 182. **(B)** John Klein drill sample in the scoop reservoir before sieving to <150 μm; Mastcam 34-mm image, sol 193. **(C)** sampled drill hole for Cumberland; MAHLI image, sol 279. **(D)** Cumberland drill sample in the scoop reservoir before sieving to <150 μm; Mastcam 34-mm image, sol 279. Borehole diameters are 1.6 cm; scoop inner width is 3.8 cm.

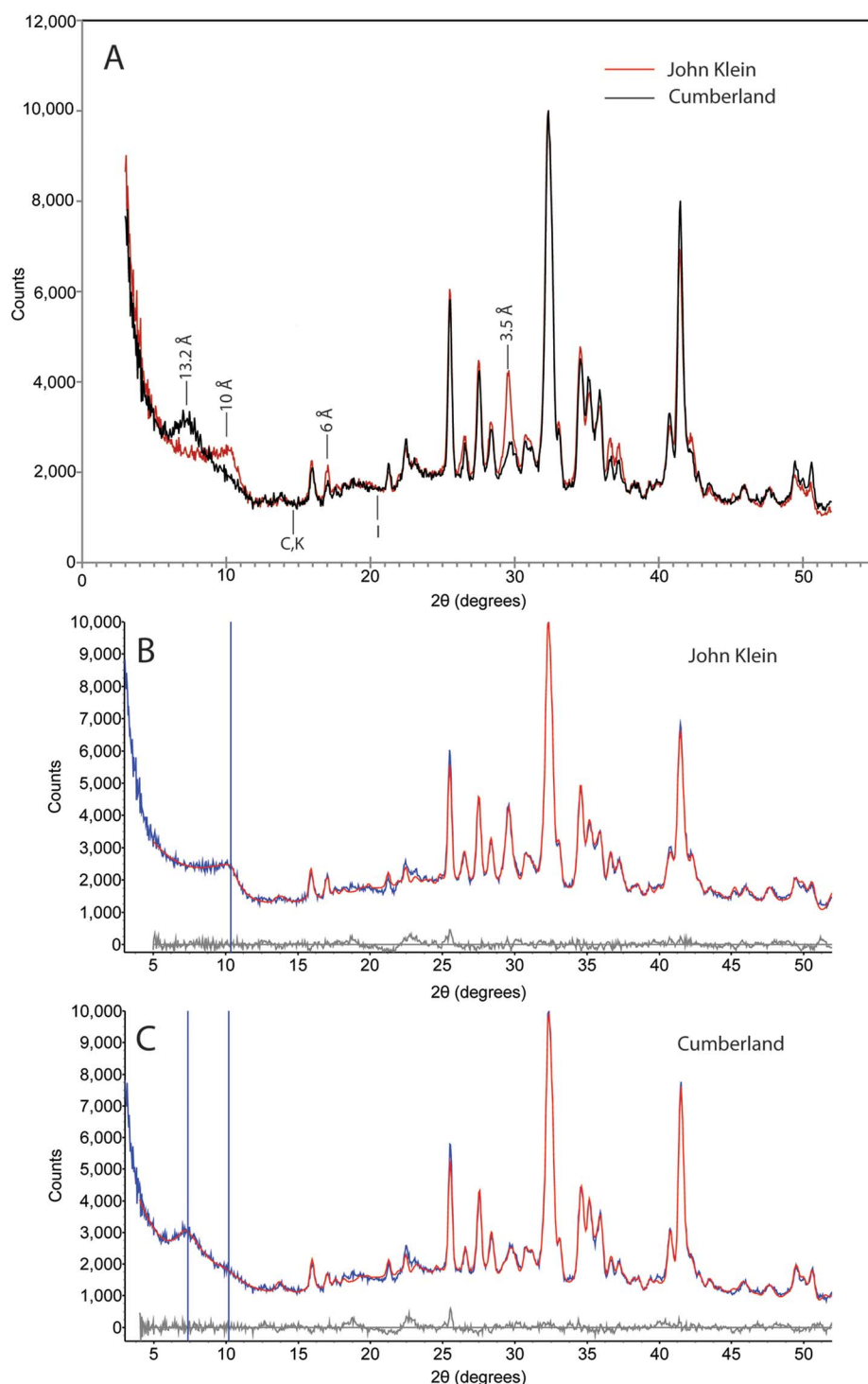


Fig. 2. X-ray diffraction patterns of mudstone samples. (A) X-ray diffraction patterns for John Klein and Cumberland superimposed. Similarity between the mudstone samples is evident, but there are notable differences in the major smectite 001 peaks at 13.2 Å in Cumberland and at 10 Å in John Klein (spacing corrected for Lorentz polarization). Also labeled are reflections at 6 Å for bassanite and 3.5 Å for anhydrite plus Fe-forsterite, which are more abundant in John Klein than in Cumberland. Letters beneath the patterns indicate where major reflections would occur for chlorite or kaolinite (C and K, at 7 Å) and illite/mica (I, at 5 Å). (B and C) Rietveld refinement results for John Klein (B) and Cumberland (C), showing observed (blue) versus calculated (red) patterns, with difference curves at the bottom (gray). Vertical scales show intensity; horizontal scales are 2θ (Co Kα). Vertical lines mark the position of the broad 001 basal reflection of collapsed smectite at 10 Å in John Klein and, in Cumberland, a prominent peak at lower 2θ (13.2 Å) with a more subdued peak at higher 2θ (10 Å).

John Klein and Cumberland left little if any Rocknest contamination in Cumberland; conversely, some of the Fe-forsteritic olivine present in the John Klein sample might be contamination from the Rocknest sample.

Phyllosilicates

CheMin XRD data reveal the presence of phyllosilicates in John Klein and Cumberland (Fig. 2A). A broad 001 diffraction peak in the John Klein XRD pattern extends from 12 to 9.4 Å, corresponding with the large interlayer spacing of a 2:1 smectite. This broad range of 001 diffraction is common to a variety of phyllosilicates, but the breadth of this peak, and the lack of other well-defined peaks (such as an 002 peak at 5 Å), argue against the presence of well-crystallized phyllosilicates such as mica or illite. Well-defined diffraction peaks for kaolinite or chlorite-group minerals at 7 Å are absent. A smectite with similar diffraction properties is present in Cumberland, although the low-angle region includes a second peak ranging from ~12 to 17 Å with a maximum at ~13.2 Å. This larger interlayer spacing in the Cumberland XRD pattern is a noteworthy characteristic.

The interlayer spacing in a smectite, revealed by the broad 001 peak, is affected by the layer charge, the nature of the interlayer cation(s) (typically K, Na, and/or Ca; less commonly Mg), the hydration state of the interlayer cations, and the possible presence of chloritic interlayers. The layer charge and interlayer cation content of a smectite are relatively stable in solid samples, so changes in interlayer spacing are mostly dependent on relative humidity. Modeling and experimental studies (28, 29) suggest that if exposed at Mars surface conditions, smectites can go through diurnal and seasonal hydration cycling, with substantial dependence of the amount of hydration on the nature of the interlayer cation. For example, Ca-smectite will hold more interlayer H₂O than Na-smectite at the same conditions (30). The John Klein and Cumberland samples inside the body of Curiosity were exposed to higher and less variable temperature (a diurnal range of 5° to 25°C) than they were in situ. These temperatures yield very low relative humidities, and dehydration should be favored. The position and breadth of the 001 diffraction peak in the John Klein sample have not changed over 30 sols of analysis following collection, but at 10 Å this smectite appears to be largely dehydrated and little or no change would be expected. The larger 001 spacing in Cumberland has also been static over 28 sols of analysis; the preservation of this wider spacing suggests a difference in the interlayer composition of the smectite in Cumberland compared with John Klein.

Possible explanations for persistent larger interlayer spacing in Cumberland include smectite having hydrated interlayers with H₂O molecules retained by high hydration-energy interlayer cations, possibly Mg²⁺ (28) or Ca²⁺ (30), and partial pillaring of the interlayer by metal-hydroxyl

groups, as with incipient chloritization (31), that would prevent collapse. The nodule-bearing portions of the mudstone that were drilled for sampling pass laterally into mudstone with early-digenetic Mg-rich raised ridges described above. These could be sources of Mg for cation exchange or incipient chloritization, focused more on Cumberland than on John Klein. Cation exchange occurs readily, with the interlayer cation largely reflecting the dominant cation in solution. Incipient chloritization by fixation of Mg-hydroxy groups can occur under surficial conditions when exposed to Mg-rich alkaline fluids, a process observed in some saline lakes (32). Hydrothermal fluids may induce this change as well (33).

X-ray diffraction analysis of clay minerals in terrestrial laboratories has the advantage of additional sample processing, such as preparation of oriented mounts, controlled variation of relative humidity, treatment with ethylene glycol, and heat treatment. These treatments are not possible in CheMin on Mars. In addition, a substantial component of smectite classification is in determination of trioctahedral or dioctahedral crystal structure (the range from full to two-thirds occupancy of sites in the octahedral sheet), but this is generally accomplished by analysis of the 06l diffraction band at ~1.54 Å (trioctahedral, ~71° 2θ Co Kα) to ~1.50 Å (dioctahedral, ~73° 2θ Co Kα). This is beyond the diffraction range of the CheMin CCD detector (~50° 2θ). However, other components of the diffraction pattern correlate similarly with this structural difference. The position of the maximum in the 02l band, at ~22.5° to 23.1° 2θ Co Kα, corresponds with the range from trioctahedral to dioctahedral structures (Fig. 3). The 02l two-dimensional diffraction band is asymmetric, is often overlapped by diffraction peaks from other phases (e.g., augite), and therefore is not as easy to measure as 06l. However, this band provides similar information, as its position is related to the b unit cell parameter in the same way as the 06l band. The 02l diffraction band maximum for the John Klein and Cumberland samples (Fig. 3) is at 22.5°, indicative of a trioctahedral clay mineral such as saponite or Fe-saponite and not of dioctahedral forms such as montmorillonite. Some Fe-bearing dioctahedral smectites such as nontronite have similar 02l bands, but the fit is not as good as with saponite.

Oxide and Sulfide Minerals

Magnetite is the prominent oxide phase in John Klein and Cumberland, as it is at Rocknest (23). Magnetite is present at 3.8 weight percent (wt%) in the John Klein sample and 4.4 wt% in Cumberland (Table 1). These abundances are significantly higher than in Rocknest (1.5 wt%). As a basic observation, concentration of detrital magnetite in sedimentary mudstones is surprising. Grains of magnetite (ρ ~ 5 g/cm³) are not expected to be enriched in very fine grained detrital sedimentary rocks otherwise composed of olivine, pyroxene, and feldspar (ρ ~ 2.7 to 3.7). By reason of hydraulic equivalence, grains of

higher density may be present but are expected to be smaller in size, and enrichment should not occur (34). Other factors are required for selective enrichment, such as free settling of grains in turbulent flow, selective entrainment of grains from a granular bed by flowing water, and shearing of grains in a moving granular dispersion (35). However, because the Sheepbed mudstone likely formed by nonturbulent settling of fines from suspension in a body of standing water (8), we expect that none of these processes would have been

influential in causing a hydraulic enrichment of heavy minerals. The relatively high abundance of magnetite in the Sheepbed mudstone may have been caused by authigenesis. Authigenesis of magnetite is further suggested by the observation that high magnetite abundance is associated with loss of Fe-forsterite and the appearance of smectites. Unit cell parameters of magnetite in the mudstone are about 0.2% smaller than for ideal magnetite, with a unit cell edge of 8.38 versus

Table 1. Crystalline and amorphous components (wt%) of the John Klein and Cumberland drill powders, compared with the Rocknest scooped eolian deposit (23). Relative 2σ errors are comparable to those cited in (22). From plagioclase to pyrrhotite the estimated errors are ~6% of the amount shown for abundances of >20%, ~15% for abundances of 10 to 20%, ~25% for abundances of 2 to 10%, and ~50% for abundances of <2% but above detection limit. Phases marked with an asterisk are at or near detection limit. Relative 2σ errors are ~50% of the amount shown for smectite and ~60% for the amorphous component.

Mineral	Rocknest	John Klein	Cumberland
Plagioclase	29.8	22.4	22.2
Fe-forsterite	16.4	2.8	0.9
Augite	10.7	3.8	4.1
Pigeonite	10.1	5.6	8.0
Orthopyroxene		3.0	4.1
Magnetite	1.5	3.8	4.4
Anhydrite	1.1	2.6	0.8
Bassanite		1.0	0.7
Quartz	1.0	0.4*	0.1*
Sanidine	0.9*	1.2	1.6
Hematite	0.8*	0.6*	0.7
Ilmenite	0.7*		0.5*
Akaganeite		1.1	1.7
Halite		0.1*	0.1*
Pyrite		0.3*	
Pyrrhotite		1.0	1.0
Smectite		22	18
Amorphous	27	28	31

Table 2. Refined unit cell parameters for some of the major crystalline phases in the Rocknest soil compared with those for the John Klein and Cumberland mudstone samples. Unit cell parameters for the augite in John Klein had large errors and therefore are not reported.

Mineral	Parameter	Rocknest	John Klein	Cumberland
Fe-forsterite	a (Å)	10.327(7)	10.323(13)	10.360(34)
	b (Å)	6.034(7)	6.048(8)	6.035(24)
	c (Å)	4.771(5)	4.793(10)	4.798(23)
	α (°)	93.43(4)	93.46(5)	93.49(4)
	β (°)	116.26(2)	116.29(2)	116.34(2)
Plagioclase	a (Å)	8.177(6)	8.183(5)	8.175(4)
	b (Å)	12.868(9)	12.891(8)	12.887(6)
	c (Å)	7.113(5)	7.127(5)	7.127(4)
	γ (°)	90.13(3)	90.03(4)	90.04(3)
	β (°)	106.25(9)	—	105.94(11)
Augite	a (Å)	9.782(9)	—	9.796(13)
	b (Å)	8.939(9)	—	8.960(13)
	c (Å)	5.269(7)	—	5.243(9)
	β (°)	106.25(9)	—	105.94(11)
	β (°)	108.0(1)	108.7(1)	108.5(1)
Pigeonite	a (Å)	9.652(9)	9.698(15)	9.698(12)
	b (Å)	8.92(1)	8.925(13)	8.925(12)
	c (Å)	5.254(7)	5.230(8)	5.230(7)
	β (°)	108.0(1)	108.7(1)	108.5(1)
	β (°)	108.0(1)	108.7(1)	108.5(1)
Magnetite	a (Å)	8.39(2)	8.384(5)	8.383(3)
	a (Å)	8.39(2)	8.384(5)	8.383(3)

8.39 Å. A possible explanation of the smaller cell size is partial (~20%) oxidation of the ferrous iron, toward the ferric defect-spinel maghemite (8.33 Å) in which some Fe sites are vacated to preserve charge balance. Alternatively, substitution of smaller cations such as Cr, Mg, or Al could account for a smaller unit cell, although it is not clear whether sufficient amounts of these are present.

In addition to magnetite, the Rietveld refinements are consistent with small amounts of ilmenite and hematite in the mudstone samples. Also present is akaganeite, β -FeO(OH,Cl), which is a possible oxide host for Cl. It has been previously suggested (36), based on mid-IR and visible-near-IR spectra, that akaganeite may be a precursor to hematite observed from orbit on Mars, but the same study concluded that goethite was a more likely precursor. However, the mudstone at Yellowknife Bay is not a typical martian surface material and the colors of the mudstone beneath its reddish dust mantle are substantially different (Fig. 1). Akaganeite was detected in both mudstone samples, but not at Rocknest. Akaganeite at its type locality on Earth (37) occurs as an alteration product of pyrrhotite, a sulfide that is also found in the Yellowknife Bay mudstone but not in the Rocknest sample (Table 1). Occurrence of this association at Yellowknife Bay may be evidence of a similar alteration relationship. Somewhat higher abundance of akaganeite in Cumberland than in John Klein (Table 1) suggests that it could be a component of concretion formation, especially of hollow nodules that appear to be twice as abundant at Cumberland as at John Klein.

Sulfate Minerals

Veins of Ca sulfate, believed to be gypsum, have been detected by the MER rover Opportunity at the western edge of Cape York on the rim of Endeavor crater (38). Calcium sulfate hydrates, including both gypsum and bassanite, have been

inferred from OMEGA and CRISM orbital spectroscopy in multiple locations on Mars (39, 40), but with a lack of hydration bands at visible-near-IR wavelengths, anhydrite has been elusive.

CheMin XRD data show that the Sheepbed mudstone contains bassanite and anhydrite (Table 1). Anhydrite was also detected in the Rocknest eolian deposit. We have found no XRD evidence for gypsum in either Rocknest or the two mudstone samples. However, Mastcam hydration index measurements are consistent with the presence of gypsum in some of the veins crossing the mudstone, showing that the vein system might contain all three of the principal Ca sulfate phases. Specifically, Mastcam's longest-wavelength filter (1013 ± 21 nm) can detect the $2\nu_1 + \nu_3$ H₂O combination absorption band and/or the 3ν OH overtone absorption band in specific hydrated minerals (16, 27, 41). Calibrated Mastcam spectra show evidence for hydration associated with some light-toned, Ca sulfate-bearing features in the Sheepbed unit, including some veins (Fig. 4, A and B) and some fillings within hollow nodules. However, the hydration signature is not universal in these light-toned features; several narrow veins observed in the John Klein vicinity show no evidence for hydration. From comparisons with laboratory reflectance spectra of Ca sulfate minerals convolved to Mastcam bandpasses (Fig. 4C), the hydration signature near 1013 nm is consistent with the presence of gypsum but not bassanite or anhydrite (24). The presence of some Ca sulfate veins that exhibit the Mastcam hydration signature and others that do not, with apparent lower hydration in thinner veins, is in accord with XRD observation in the drill samples of bassanite and anhydrite but not gypsum.

Before the John Klein drill sample was collected, observations by LIBS, supported by APXS analyses of some veins, had indicated widespread association of Ca and S in light-toned veins and filling hollow nodules in Yellowknife Bay. The LIBS and APXS data and Mastcam spectral interpretations suggest hydrogen associated with some but not all of these light-toned materials. The drill

locations for John Klein and Cumberland were deliberately targeted to collect samples of the mudstone matrix with as little sulfate veining as possible (Fig. 1, A and C). Nevertheless, hair-line fractures and fillings within hollow nodules were observed on borehole walls (24), and these are likely the principal or sole hosts of Ca sulfate minerals in the John Klein and Cumberland samples.

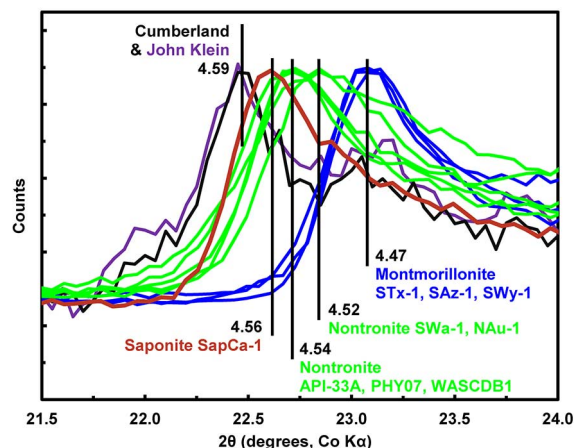
Bassanite does not have a stability field at pressures less than 235 MPa (42), far in excess of the maximum pressure (~50 MPa) that would be attained if the Sheepbed mudstone had been buried under ~5 km of sediment (a possibility because the mudstone could be exhumed from beneath the 5-km-high stratigraphy of Mt. Sharp). Bassanite in the mudstone is not in equilibrium, but it may persist for long periods because of the unique surface conditions on Mars. Bassanite is relatively rare on Earth because it readily hydrates to form gypsum, even at low relative humidity. However, the very low vapor pressure of H₂O in the atmosphere of Mars may favor persistence of bassanite (43, 44). Although nominal near-equatorial surface conditions are unlikely to desiccate gypsum to form bassanite (44), moderate increase in temperature or decrease in partial pressure of H₂O could lead to destabilization of gypsum and formation of bassanite (45, 46).

Bassanite forms in many different ways on Earth. Examples include dissolution-reprecipitation after gypsum in sabkha environments (47), gypsum dehydration in endoevaporitic microbial communities under slightly alkaline conditions (48), alteration of carbonates in acid-sulfate systems (49), and dehydration of gypsum dunes (50) or arid sedimentary rocks (51) in desert environments. Bassanite of undetermined origin also occurs along with gypsum in soil of the Transantarctic Mountains (52). In most of these bassanite occurrences on Earth, the associated or precursor Ca sulfate is gypsum because bassanite is often a product of gypsum dehydration. In these representative studies, association of bassanite with anhydrite, as in the John Klein sample, does not occur and is apparently rare. This is probably

Table 3. Compositions of major crystalline phases in the Rocknest soil compared with those for the John Klein and Cumberland mudstone samples, based on unit cell parameters in Table 2.

Rocknest	
Fe-forsterite	(Mg _{0.62(3)} Fe _{0.38}) ₂ SiO ₄
Plagioclase	(Ca _{0.57(13)} Na _{0.43})(Al _{1.57} Si _{2.43})O ₈
Augite	(Ca _{0.75(4)} Mg _{0.88(10)} Fe _{0.37})Si ₂ O ₆
Pigeonite	(Mg _{1.13(9)} Fe _{0.68(10)} Ca _{0.19})Si ₂ O ₆
John Klein	
Fe-forsterite	(Mg _{0.51(5)} Fe _{0.49}) ₂ SiO ₄
Plagioclase	(Ca _{0.44(12)} Na _{0.56})(Al _{1.44} Si _{2.56})O ₈
Pigeonite	(Mg _{1.08(12)} Fe _{0.82(7)} Ca _{0.10})Si ₂ O ₆
Cumberland	
Fe-forsterite	(Fe _{0.54} Mg _{0.46(12)}) ₂ SiO ₄
Plagioclase	(Ca _{0.43(11)} Na _{0.57})(Al _{1.43} Si _{2.57})O ₈
Augite	(Ca _{0.82(5)} Mg _{0.68(13)} Fe _{0.50})Si ₂ O ₆
Pigeonite	(Mg _{1.08(11)} Fe _{0.80(6)} Ca _{0.12})Si ₂ O ₆

Fig. 3. Comparison of 02l diffraction bands of John Klein and Cumberland with other smectites. The 2θ range is selected to contain the maximum intensity positions (in Å) and profiles of the 02l diffraction bands. Smectites used for comparison include a range of trioctahedral (e.g., saponite SapCa-1) to dioctahedral (e.g., montmorillonite STx-1) smectites.



because temperatures of anhydrite formation are generally high enough not to favor a metastable bassanite precursor, and hydration of anhydrite is likely to go directly to gypsum.

Anhydrite is a common mineral on Earth, although it hydrates to form gypsum in sufficiently humid environments. Hydration rates for “soluble anhydrite” (having remnant channel structure similar to bassanite) are relatively rapid; hydration rates are much slower for insoluble anhydrite (53). Where activity of pore waters is above ~0.9 and up to 1.0, anhydrite is the stable Ca sulfate mineral at burial depths where temperatures rise above ~50° to 58°C [e.g., (54)]. The temperature of this transition decreases as H₂O activity decreases, and thus the occurrence of anhydrite, in the absence of other information, can be a poor guide to past temperatures. However, if an anhydrite occurrence carries other information that constrains the activity of water, it is a reasonable indicator of elevated temperature. Moreover, persistence of anhydrite, as of bassanite, indicates a lack of postformation hydration.

Low-pH acid-sulfate weathering has long been proposed for many locations on Mars [e.g., (55)]. Acid-sulfate weathering was likely to have been much more pervasive in the Noachian, when major impacts had substantial influence on hydrosphere chemistry (56). In the well-studied Burns formation of Meridiani Planum, the occurrence of Fe sulfate phases such as jarosite is evidence of such conditions, with diagenesis related to persistent groundwater of high ionic strength (57). The absence of Fe sulfates at John Klein and Cumberland, and the presence of Ca sulfates in

stead, is evidence of an environment with low ionic strength and circumneutral pH.

The X-ray Amorphous Component

The amorphous component of the mudstone may represent soil or eolian fines accumulated along with crystalline detritus in the mudstone, but the nature and origin of the amorphous component is poorly known. Estimated composition of the amorphous component in the mudstone (24) varies depending on the assumed composition of the phyllosilicates but generally indicates a relatively Si-poor material enriched in Fe, S, Cl, and P. The estimated compositions of amorphous material in the mudstone are approximately similar to the amorphous component of the Rocknest eolian deposit (24, 25), but possibly modified during diagenesis in the mudstone, including smectite formation and subsequent cation exchange or other interlayer adjustments.

Implications of the Sheepbed Mudstone Mineral Assemblage

Detrital plagioclase, clinopyroxenes, and Fe-forsterite identified by CheMin are generally similar in composition for Rocknest and the mudstone samples John Klein and Cumberland (Table 3). This suggests a common basaltic source for much of the crystalline detritus in both the eolian and mudstone samples. The abundance of magnetite relative to other crystalline phases in the mudstone, however, is in excess of what would be expected for likely basaltic source rocks; normalized to the igneous detrital minerals the magnetite abundance rises from 2.1 wt% in Rocknest to 8.7 wt%

in John Klein and 9.5 wt% in Cumberland. Abundant magnetite in the mudstone could indicate either authigenic formation or a mechanism of sedimentary accumulation. The XRD data alone cannot distinguish between these origins, but the mudstone sedimentary context (8) argues against detrital accumulation of heavy minerals.

Occurrence of gypsum, bassanite, and anhydrite in veins transecting the Yellowknife Bay formation is a disequilibrium association. Persistence of bassanite and anhydrite places limits on post-diagenesis hydration. The Sheepbed mudstone mineralogy favors both formation and preservation of the markers of habitability, having been formed in an aqueous depositional environment with late diagenesis limited to fractures that are isolated from the sediment matrix and with little or no evidence of hydrous alteration following late diagenesis.

The phyllosilicate in John Klein is trioctahedral and likely a saponitic smectite. The clay mineral in Cumberland appears to be genetically related, with an almost identical 02/ band, although its interlayer constituents are different. The greater basal spacing of the Cumberland smectite may reflect intercalation of Mg-hydroxy interlayers. Tendency toward interlayer modification may be widespread on Mars, as indicated by spectral studies that point to the common occurrence of smectite/chlorite mixed-layer clay minerals (58).

Smectites in the mudstone could be detrital, neoformed, or formed from primary phases by authigenic alteration (59). Any of these origins could be compatible with a habitable environment. Relative to other basaltic detrital minerals, Fe-forsterite

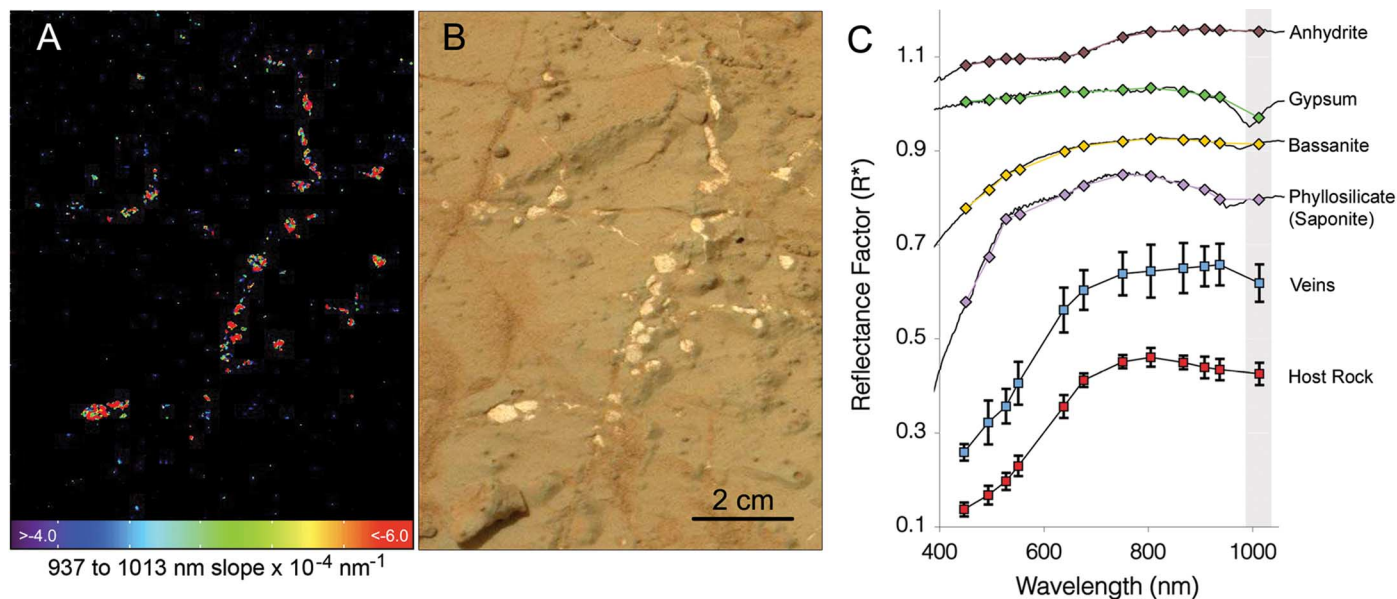


Fig. 4. Mastcam “hydration signature” data for veins in the Sheepbed unit at the target “Knorr” acquired on sol 133, sequence mcam00805. (A) Colors indicate regions where the 937- to 1013-nm spectral slope is negative and below a threshold consistent with the spectra of many hydrated minerals (24). **(B)** Mastcam R0 color image of the Knorr veins at the same scale as in (A). **(C)** Comparison of Mastcam relative reflectance [R^* (24)] spectra of the Knorr veins and host rock with laboratory reflectance spectra (65) of three

Ca sulfates with different states of hydration and a representative hydroxylated phyllosilicate (saponite). Solid lines are full-resolution lab spectra; diamonds indicate lab spectra values convolved to Mastcam bandpasses; vertical gray line indicates location of Mastcam hydration band. The anhydrite and gypsum data are offset by +0.25 and +0.1 reflectance units, respectively. Error bars in the Mastcam spectra represent the standard deviations of the group of pixels sampled.

is disproportionately reduced in John Klein and is almost absent in Cumberland. Assuming an initial presence of olivine in proportions consistent with typical martian basaltic compositions [as at Rocknest (9)], the loss of Fe-forsterite in the mudstone is likely to be a consequence of alteration during authigenic formation of clay minerals. This conclusion is supported by evidence of isochemical alteration (19) as well as evidence of diminished Fe-forsterite abundance associated with proportional increase in magnetite and appearance of clay minerals (Table 1). Analogous alteration of Fe-forsterite is the central process in forming saponitic, trioctahedral clay minerals plus magnetite in chondritic meteorites at temperatures of <100°C (60). In the Sheepbed mudstone this process may be related to concretion formation, perhaps associated also with formation of akaganeite. Consequences of such a reaction could include lower Eh (oxidation potential), higher pH that favors intercalation of Mg-hydroxy interlayers in the clay minerals, and possibly production of H₂ gas that might account for the voids in the hollow nodules. This scenario of Fe-forsterite “saponitization” is conjectural but worth consideration. The possible formation of H₂ gas as part of this process could be another component of habitability, providing a potential energy source for chemolithoautotrophs.

The clay mineral in John Klein has a diffraction pattern suggestive of a smectite that retains swelling capacity, but the signature of the clay mineral in Cumberland is less definitive. Indeed, the larger basal spacing of the clay mineral in Cumberland suggests that it is either hydrated or expanded by some form of intercalation. Furthermore, the persistence of hydration over 30 sols in the warm body of the rover (5° to 25°C) at very low relative humidity is unlikely, so we favor the interpretation of a structural modification. Differences in clay mineralogy over such a short distance between two samples indicate variable diagenetic modification in a mineralogically immature sedimentary rock.

The lack of collapsed and highly ordered illite or chlorite in the Sheepbed member mudstone argues against prolonged, deep burial at elevated temperature. In terrestrial shales, development of corrensite or chlorite generally requires alteration temperature in excess of ~60° to 80°C [e.g., (61)]. Absence of such clay mineral modification, beyond the proposed incipient chloritization and partial intercalation of Mg-hydroxy interlayers in clay minerals of the Cumberland sample, suggests alteration at temperatures lower than this. This is a fairly loose constraint at Gale crater, as complete burial of the crater may have resulted in a maximum burial temperature of only ~75°C (62). As noted above, formation of late-diagenetic anhydrite from solutions of low salinity (19) may indicate temperatures above ~50°C; however, these solutions probably originated at depth from zones at higher temperature. In summary, evidence from the mudstone mineralogy supports modest authigenesis temperatures but does not constrain depth of burial.

The preponderance of clay mineral formation on Mars, with associated habitable environments, has been attributed to Noachian processes (63). Estimated ages for the Sheepbed mudstone are poorly constrained, but sediments in the Gale crater mound are no older than Late Noachian/Early Hesperian (64) and the Yellowknife Bay formation is likely no older than Early Hesperian (8). The Sheepbed member provides an example of an environment where clay mineral formation continued to occur beyond the end of the Noachian Epoch.

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Acknowledgments: This paper was significantly improved by comments and recommendations from two anonymous reviewers. Support from the NASA Mars Science Laboratory Mission is gratefully acknowledged. Some of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.

Supplementary Materials
www.sciencemag.org/content/343/6169/1243480/suppl/DC1
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19 July 2013; accepted 29 October 2013
 Published online 9 December 2013;
[10.1126/science.1243480](https://doi.org/10.1126/science.1243480)

Elemental Geochemistry of Sedimentary Rocks at Yellowknife Bay, Gale Crater, Mars

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Sedimentary rocks examined by the Curiosity rover at Yellowknife Bay, Mars, were derived from sources that evolved from an approximately average martian crustal composition to one influenced by alkaline basalts. No evidence of chemical weathering is preserved, indicating arid, possibly cold, paleoclimates and rapid erosion and deposition. The absence of predicted geochemical variations indicates that magnetite and phyllosilicates formed by diagenesis under low-temperature, circumneutral pH, rock-dominated aqueous conditions. Analyses of diagenetic features (including concretions, raised ridges, and fractures) at high spatial resolution indicate that they are composed of iron- and halogen-rich components, magnesium-iron-chlorine-rich components, and hydrated calcium sulfates, respectively. Composition of a cross-cutting dike-like feature is consistent with sedimentary intrusion. The geochemistry of these sedimentary rocks provides further evidence for diverse depositional and diagenetic sedimentary environments during the early history of Mars.

Shortly after leaving its landing site at Bradbury Landing in Gale crater, the Mars Science Laboratory Curiosity rover traversed to Yellowknife Bay (1), where it encountered a flat-lying, ~5.2-m-thick succession of weakly indurated clastic sedimentary rocks ranging from mudstones at the base to mainly sandstones at the top (2). Stratigraphic relationships and sedimentary structures indicate that this coarsening-upward succession likely represents sedimentation in an ancient fluvio-lacustrine system that would have been habitable. Also preserved is a spectrum of diagenetic features, including concretions; void spaces with a variety of sizes, geometries, and origins; early diagenetic fractures (“raised ridges”) filled with banded (possibly silicate) cements; possible sedimentary dikes; and a later diagenetic fracture system filled with sulfate cements. All of these features indicate extended postdepositional aqueous fluid flow through the rocks (2).

Curiosity fully applied its analytical payload to investigate these sedimentary rocks and determine lithological, textural, chemical, mineralogical, and isotopic compositions and their stratigraphic relationships (2–4). The payload of Curiosity includes two instruments capable of measuring elemental abundances. The alpha particle x-ray spectrometer (APXS) determines abundances on ~2.25 cm² surfaces and, when used in conjunction with the dust removal tool (DRT) or drilling system, can analyze relatively clean surfaces and

drill cuttings. The laser-induced breakdown spectrometer (LIBS), part of the ChemCam remote sensing suite, detects atomic emission spectra from areas of ~0.1 to 0.3 mm² (depending on standoff distance), more than two orders of magnitude smaller than APXS. LIBS offers the additional capability of laser depth profiling (including surface dust removal) of up to ~1000 μm. These instruments provide complementary data by revealing both bulk rock compositions and compositions that can be related directly to textural features.

On Earth, the elemental geochemistry of clastic sedimentary rocks provides information central to interpreting sedimentary history, including chemical weathering history, nature and composition of the sediment provenance, sediment transport, and diagenetic history (5–10). In turn, geochemical understanding of these issues constrains many fundamental geological questions such as paleoclimates, tectonic relationships, basin evolution, diagenetic fluid flow, and even crust-mantle evolution (7, 11–15). Findings from instruments onboard the Mars Exploration Rovers Spirit and Opportunity, orbital spectroscopy, and experiments using Mars-like compositions show that such approaches are applicable to sedimentary environments on Mars, where primary igneous compositions and aqueous conditions may differ from our terrestrial experience (12, 16–24). Accordingly, we report elemental

geochemistry of the ancient sedimentary rock succession, including its diagenetic features, preserved at Yellowknife Bay; evaluate these compositions in terms of the sedimentary history of this habitable paleoenvironment within Gale crater; and place them within the context of martian surface environments early in the planet’s history.

Yellowknife Bay Stratigraphy

The geology, stratigraphy, sedimentology, and diagenetic history of Yellowknife Bay are described in (2). The sedimentary sequence, informally named the Yellowknife Bay formation, is subdivided into three members (Fig. 1) that likely were deposited in a prograding alluvial fan fluvio-lacustrine depositional system and influenced by at least two distinct diagenetic events. The formation is likely Hesperian in age but poorly constrained and could lie in a range from early Hesperian (~3.7 to 3.4 billion years ago), if part of the early Gale crater fill, to late Hesperian or early Amazonian (~3.4 to 1.5 billion years ago),

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Exploring Martian Habitability

if coeval with the nearby Peace Vallis alluvial fan exposed in Gale crater (2).

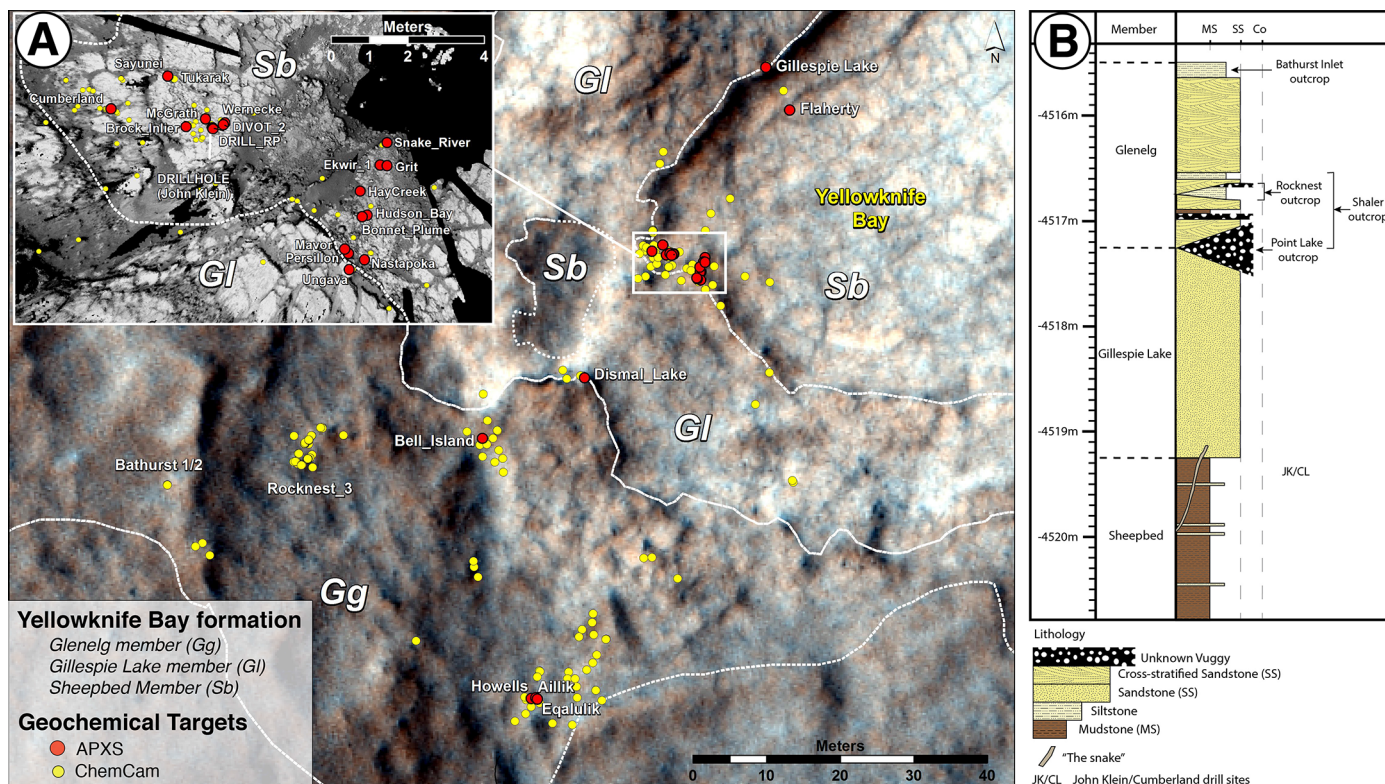
The stratigraphically lowest Sheepbed member (>1.5 m thick), whose base is not exposed, is composed of gray-colored, bedded mudstone. Fine-grained texture, laterally extensive decimeter-scale bedding, and stratigraphic relationships indicate deposition from suspension, likely in a distal alluvial fan lacustrine (or less likely playa) environment but possibly as ash fall. Early diagenetic textures, which were formed before or during lithification, include millimeter-scale nodules interpreted as concretions, millimeter-scale rimmed “hollow nodules” interpreted as void space (possibly formed by gas expulsion during early diagenesis), and narrow centimeter-sized intersecting “raised ridges” laterally correlative to nodules and hollow nodules. These ridges, whose margins are lined by resistant isopachous cements, are interpreted as diagenetic cracks formed in early lithified sediment, probably by reactions involving pore fluids [see figures 6, A to C, and 7 in (2)]. The upper ~50 to 75 cm of the Sheepbed member weathers more recessively than underlying beds and possesses higher abundances of both early and late diagenetic features. For geochemistry, we further subdivided the Sheepbed member into lower and upper parts with the boundary ~30 cm beneath the

Gillespie Lake member to evaluate compositional transitions into the overlying stratigraphic unit.

The Gillespie Lake member is ~2.0 m thick and overlies the Sheepbed member with a sharp, likely erosional, contact. It consists of thin- to medium-bedded, medium- to very coarse-grained sandstones with relict centimeter-scale cross-bedding. Mars Hand Lens Imager (MAHLI) and ChemCam Remote Micro-Imager (RMI) images indicate textural immaturity and compositionally diverse grains [see figure 5D in (2)]. This member was likely deposited in a distal fluvial environment. Gillespie Lake sandstones exhibit little primary porosity, suggesting a cemented rock, but may contain secondary porosity in the form of dispersed millimeter-scale vugs that may result from leaching of detrital grains or early diagenetic phases during fluid circulation or perhaps selective loss of readily degraded mudstone intraclasts during weathering (2). The Sheepbed (including early diagenetic features) and Gillespie Lake members are both cross-cut by a network of later diagenetic (post-lithification) intersecting fractures of variable thickness (hairline to ~8 mm) filled with light-toned cement, identified as Ca sulfate by a variety of measurements (see below). These filled fractures are most abundant within the uppermost Sheepbed member, and the bright cements also fill hollow nodules, where they

intersect with fractures. Also cross-cutting the upper Sheepbed member is a ~8-cm-wide dike-like feature termed the “snake,” that terminates in a small anticline within the middle Gillespie Lake member, and is interpreted as a sedimentary dike injected as a result of high pore pressures that developed during rapid burial (2). A detailed 1.5-m stratigraphic section, termed the Selwyn Section, was measured across the Sheepbed–Gillespie Lake contact (Fig. 1 and fig. S1).

The upper Glenelg member (~1.7 m thick) is lithologically heterogeneous (2). The lower ~40 cm (e.g., Point Lake outcrop) is poorly understood but possesses abundant millimeter- to centimeter-scale voids. Possible interpretations include a debris flow, a volcanoclastic layer, a gas-charged sedimentary sill (perhaps related to the “snake”), or a vesicle-rich volcanic flow (although there is no evidence for a nearby contemporaneous volcanic source). Some voids and cross-cutting fractures are filled with light-toned minerals, identified as Ca sulfate by ChemCam, reminiscent of fracture fillings in the Sheepbed and Gillespie Lake members. We assume that this part of the Glenelg is also sedimentary but are mindful of this uncertainty. Other parts of the Glenelg member (e.g., Shaler outcrop) consist of commonly cross-bedded interstratified sandstones and recessive finer grained sediment and was likely



deposited mainly by fluvial processes, with paleocurrents indicating a source derived from the direction of the crater rim. At the top of this member are discontinuous fine-grained beds (e.g., Bathurst Inlet), in some cases with distinctive chemical compositions and possibly with distinctive origins (see below).

Mineralogical and Geochemical Constraints from CheMin and SAM

Two locations within the upper Sheepbed member, corresponding to APXS targets John_Klein and Cumberland, were drilled, with recovered powders being sieved (<150 μm) and delivered to CheMin and SAM (Sample Analysis at Mars) for x-ray diffraction (XRD) analysis of mineralogy and chemical-isotopic measurements of evolved gases using gas chromatography, quadrupole mass spectrometry, and tunable laser spectrometry (3, 4). Unsurprisingly for clastic sediments, both samples are composed of nonequilibrium mineral assemblages including primary igneous and a variety of secondary phases, notable for a ~30 to 40% amorphous component (including allophane-like material) and ~20% trioctahedral phyllosilicates. The remaining crystalline mineralogy includes (in decreasing amount) plagioclase, pyroxene, magnetite, Ca sulfate (anhydrite, bassanite), forsteritic olivine (John_Klein only), akaganeite, sanidine, pyrrhotite, and possibly (<1 to 2%) hematite, ilmenite, pyrite, quartz, and halite. The exact mineralogy of the trioctahedral phyllosilicates is not fully constrained, but they consist of a ~10 Å collapsed saponitic smectite in John_Klein and, in Cumberland, clay minerals with both ~10 Å and ~13.2 Å spacing, possibly reflecting poorly formed vermiculite (lacking the 7 Å peak) or a smectite with either an interlayer cation with high hydration energy (e.g., Mg) or metal-hydroxy groups (i.e., incipient chlorite). SAM analyses are also consistent with smectite, sulfates, sulfides, and Fe oxides; oxychlorine compounds (e.g., chlorate and/or perchlorate) are indicated as well. Both instruments indicate heterogeneous Fe and S redox states. Mass balance calculations provide estimates of the chemical compositions of the combined amorphous component plus phyllosilicates and remaining crystalline minerals, and these compositions (4) are used below.

Clastic Sedimentary Rocks

Yellowknife Bay S- and Cl-free compositions (all on weight percent basis) determined by APXS (25) (tables S1 to S4) correspond to iron-rich basalt (i.e., mostly <48% SiO_2 , mostly >20% FeO_T , ~8 to 10% MgO , ~200 to 900 ppm Ni). Alkalis are variable (~3 to 6% $\text{Na}_2\text{O} + \text{K}_2\text{O}$; $\text{K}_2\text{O}/\text{Na}_2\text{O}$ ratio ≈ 0.2 to 1.3). CaO is highly variable (~5 to 25%), but values above ~7% correspond to high SO_3 , indicative of elevated levels of Ca sulfate. Typical for the martian surface, both SO_3 and Cl are mostly elevated in APXS observations, in part because of surface soil and dust contamination. On brushed surfaces, APXS measurements of SO_3 are as low

as 0.9% (Werneke_Brush, the analysis on the brushed surface of the Werneke target), which suggests that the sedimentary rocks may have no more than 1 to 2% SO_3 on average. On the other hand, Cl abundances are higher than in most martian soils (26), reaching 1.9% and commonly leading to low S/Cl ratios. Elevated Cl abundances ($\geq 1\%$) are likely a primary rock feature rather than just resulting from soil or dust coatings, consistent with the presence of oxychlorine compounds, akaganeite, and possibly halite (3, 4).

Up to 9% magnetite in the crystalline component of Sheepbed mudstone drill samples was detected by XRD (4). We cannot completely exclude the possibility that very fine-grained magnetite was delivered as part of the detrital load into a lake environment, but on balance the geochemistry suggests that this magnetite is not detrital. For example, there are no systematic relationships between the FeO_T/MgO ratio and FeO_T abundance that could be explained by magnetite variations; no evidence from XRD (4) for enrichments of other minerals that might be expected to form part of a heavy mineral suite (e.g., spinels) nor correlations with related structural elements of these minerals (e.g., Cr, Ti); and no geochemical evidence, from APXS or ChemCam, for detrital magnetite enrichments (e.g., Fe enrichment) in immediately overlying Gillespie Lake sandstones, where heavy minerals might be expected to concentrate even more. Absence of geochemical evidence for detrital magnetite is also consistent with deposition of the Sheepbed mudstones from suspension into a lake, where heavy mineral concentrations are unlikely (2, 4). Thus, magnetite is most likely a diagenetic phase rather than a detrital component.

Geochemical relationships are well illustrated in ternary diagrams plotting mole fractions $\text{Al}_2\text{O}_3\text{--}(\text{CaO} + \text{Na}_2\text{O})\text{--}\text{K}_2\text{O}$ (i.e., A-CN-K) and $\text{Al}_2\text{O}_3\text{--}(\text{CaO} + \text{Na}_2\text{O} + \text{K}_2\text{O})\text{--}(\text{FeO}_T + \text{MgO})$ (i.e., A-CNK-FM) (Fig. 2). On these diagrams, igneous minerals plot on or below the plagioclase-K-feldspar (A-CN-K) and feldspar-FM (A-CNK-FM) joins, whereas secondary clay minerals plot above (27). The chemical index of alteration (CIA), defined as $100 \times [\text{A}/(\text{A} + \text{C} + \text{N} + \text{K})]$, reveals any chemical weathering history by quantifying the systematic loss of relatively mobile elements (i.e., Ca, Na, K) from silicate minerals, and the scale is plotted beside the A-CN-K diagrams. For mafic sources, CIA values above ~40 to 45 in bulk sediment suggests some chemical weathering history, and values above ~50 to 55 provide fairly compelling evidence for open system weathering (10). ChemCam (Fig. 2, C and D) analyzed many more targets than APXS (Fig. 2, A and B), and given the strategy of using its greater spatial resolution to target textural features (e.g., veins, ridges, grains), more scatter is both expected and observed. Mission experience so far indicates a slight instrument bias with ChemCam data plotting at higher Al_2O_3 on these diagrams than APXS, especially for alkali-rich compositions. Nonethe-

less, the analyses provide consistent geochemical trends.

Lower Sheepbed and Gillespie Lake bulk rock compositions (Fig. 2, A and B) are very tightly grouped and are slightly more mafic than average martian crust (closer to FM apex). Most upper Sheepbed samples plot in a similar position, but several trend toward the CN and CNK apexes, where Ca sulfate plots. ChemCam analyses (Fig. 2, C and D) show a more complete linear trend, with one end member defined by Ca sulfates within the light-toned fractures and filled hollow nodules that were targeted for analysis. The Glenelg member has bulk compositions that commonly differ from both average crust and Sheepbed/Gillespie Lake, with less relative amounts of $\text{FeO}_T + \text{MgO}$ and higher K_2O in some samples; these characteristics were confirmed by ChemCam analyses, which also showed evidence for detrital feldspar (Fig. 2D). An analysis of APXS data for the Yellowknife Bay formation samples Bathurst Inlet and Rocknest3, as well as the float rocks Jake_Matijevic (Jake_M) and Et_Then on Bradbury Rise (which have been inferred to be volcanoclastic or igneous), shows that the elemental relationships among these rocks are consistent with physical mixing between Bathurst Inlet-like and Jake_M-like material with addition of an Fe-rich cement or rind, especially apparent in Et_Then (28). Some ChemCam analyses plot above the plagioclase-K-feldspar and feldspar-FM joins, suggesting phyllosilicate-rich targets, consistent with the identification of phyllosilicates by XRD (4). In addition, some ChemCam Sheepbed analyses plot closer to the FM apex, consistent with the identification of Mg-Fe-Cl-rich phases associated with raised ridges (also observed in the McGrath APXS raster) that were also targeted for analysis (see below) and the Fe^{3+} -rich phases identified by XRD (e.g., magnetite, akaganeite, and possibly hematite).

Despite identifying phyllosilicates in Sheepbed mudstones by XRD (4) and inferring them from ChemCam (Fig. 2, C and D), the geochemistry of the Yellowknife Bay formation provides scant support for any substantial chemical weathering history affecting the sources or the sediment during transport into the depositional basin. During circumneutral pH weathering, clay minerals form at the expense of primary igneous phases, with loss of mobile elements. As a result, bulk sedimentary rock compositions that have been influenced by weathering, including those derived from mafic sources, typically plot above the plagioclase-K-feldspar and feldspar-FM joins (29–31). Similarly, any clay minerals that formed by hydrothermal alteration (e.g., impact-related) in surrounding regions and transported into the Yellowknife basin would also carry comparable distinct chemical signatures (22, 32–34). Nor is any evidence observed for low-pH alteration conditions in the form of Fe^{3+} mobility and associated formation of Fe^{3+} sulfates, such as that observed by the Spirit and Opportunity rovers (35–37). During transport, clays would concentrate more in fine-grained

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sediment (i.e., Sheepbed) than in associated coarse-grained sediment (i.e., Gillespie Lake), but no evidence for the predicted geochemical fractionation related to hydraulic segregation of sediment

types (including clay minerals and magnetite) is observed in the bulk analyses of either unit.

One complicating factor is that secondary Ca sulfate might lower the apparent CIA values and

thereby mask evidence for chemical weathering (27). However, on a plot of CIA versus SO_3 content (Fig. 3), this clearly is not the case. At high SO_3 , indicative of sulfates, CIA falls to lower

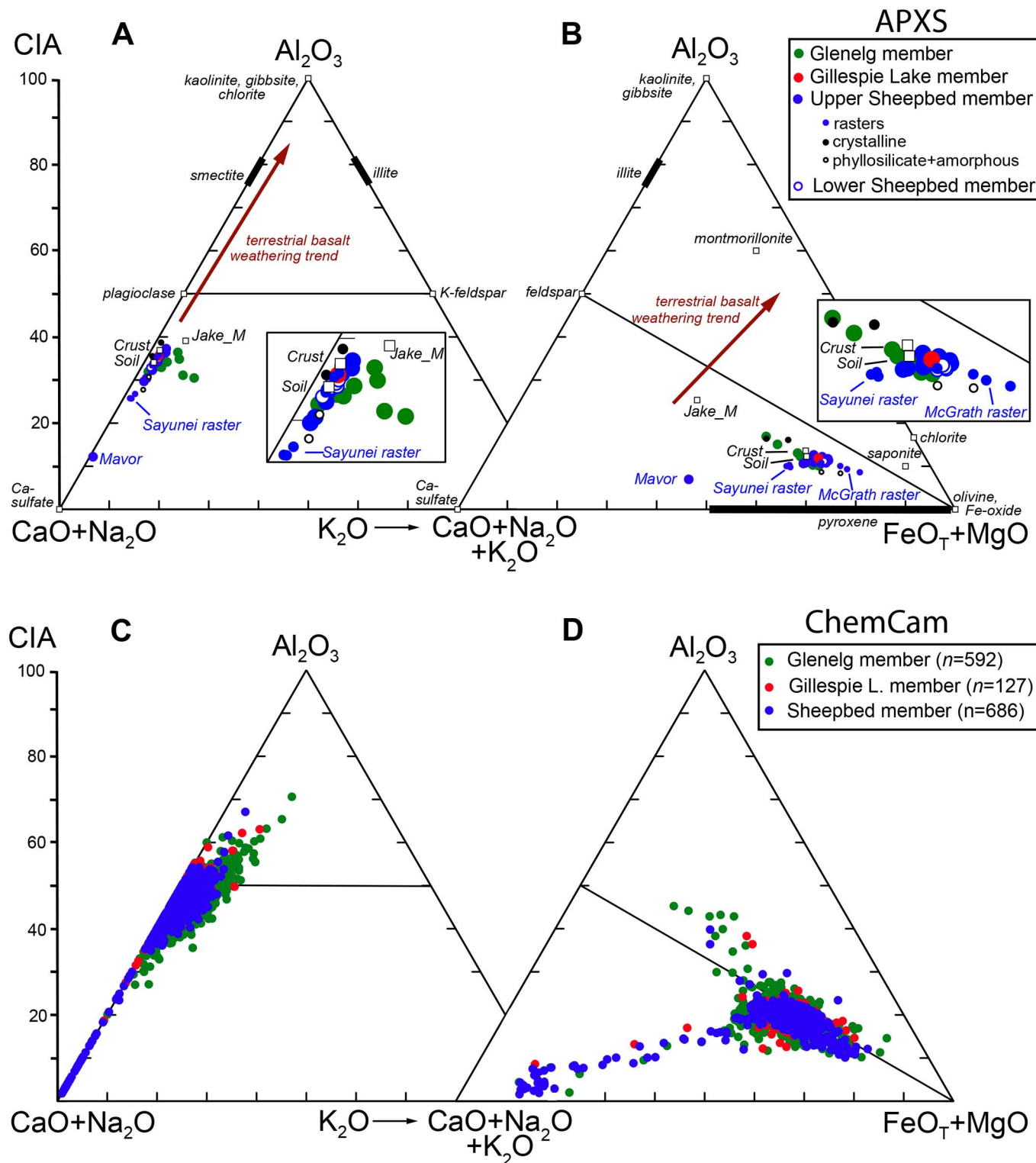


Fig. 2. Al_2O_3 -($\text{CaO} + \text{Na}_2\text{O}$)- K_2O and Al_2O_3 -($\text{CaO} + \text{Na}_2\text{O} + \text{K}_2\text{O}$)-(FeO_T + MgO) ternary diagrams. (A and B) APXS data; (C and D) ChemCam data. Shown for reference are the CIA scale [measured on (A) and (C) only], and, as open squares, average martian crust (12), local soil (64), and the local rock

Jake_Matijevec (Jake_M) (45), modeled compositions of John_Klein and Cumberland crystalline and clay + amorphous materials (4), and arrows representing typical trends observed for terrestrial weathering profiles on basalts (65). The insets of (A) and (B) are expanded views of the main cluster of data.

values as expected, whereas at lower SO_3 , CIA levels off to values typical of martian mafic igneous rocks; such findings indicate that none of these samples has witnessed a substantial chemical weathering history. This is also consistent with sample positions on the A-CNK-FM diagram (Fig. 2B). Accordingly, formation of phyllosilicates within Sheepbed mudstones likely resulted from diagenetic processes that did not noticeably influence bulk rock composition, implying rock-dominated (i.e., low water/rock ratio) post-depositional aqueous fluid conditions.

Both major and trace elements exhibit stratigraphic trends (Fig. 4). To avoid variations in absolute abundances imparted by simple dilution effects of Ca sulfate, we plotted ratios among elements most often associated with siliciclastic components. On a plot of $\text{K}_2\text{O}/\text{Al}_2\text{O}_3$ versus $\text{TiO}_2/\text{Al}_2\text{O}_3$ (Fig. 4A), the Glenelg member includes samples with higher $\text{K}_2\text{O}/\text{Al}_2\text{O}_3$ and generally slightly more variable $\text{TiO}_2/\text{Al}_2\text{O}_3$ relative to samples from the Sheepbed and Gillespie Lake members. The two analyses from the rock Bathurst Inlet, from the top of the Glenelg member, are especially distinctive in having the highest $\text{K}_2\text{O}/\text{Al}_2\text{O}_3$ ratios. These differences are interpreted to represent a notable change in the provenance from which sediment particles were derived to include more alkali-rich basalts higher in the stratigraphic section.

Subtle differences in bulk composition also exist within the Sheepbed member itself, with the upper part of the member having slightly lower and more variable $\text{TiO}_2/\text{Al}_2\text{O}_3$ ($x = 0.104$, $\text{SD} =$

0.011) than the lower part ($x = 0.116$, $\text{SD} = 0.006$). This could also be a subtle provenance effect (38, 39), although relationships between $\text{TiO}_2/\text{Al}_2\text{O}_3$ ratio and overall bulk composition are complex in detail, especially because all compositions are basaltic (12, 40). In addition, small variations in plagioclase content that are ultimately related to sedimentary sorting effects (24) could also play a role in changing the $\text{TiO}_2/\text{Al}_2\text{O}_3$ ratio (Fig. 4A).

Stratigraphic variations in APXS trace elements are summarized on a plot of Cr/Ni versus Zn/Ni (Fig. 4B). In this case, the Glenelg member has higher and/or more variable ratios than the Sheepbed member, related to combinations of elevated Zn and Cr and lower Ni. There is also a difference between the Gillespie Lake sandstone and Sheepbed mudstones, related mainly to lower Ni in the former, although only a single high-quality analysis is available for the Gillespie Lake member. Major elements indicate a similar provenance, so the reason for this is not clear. A heavy mineral effect is one possibility but would be more likely to cause an enrichment of Cr (chromite) rather than depletion of Ni in the sandstones. Thus, in Glenelg sandstones, Ni is similarly low (~200 to 400 ppm) as in the Gillespie Lake sandstone sample, but Cr reaches very high levels (>5000 ppm) consistent with a heavy mineral (chromite) enrichment predicted by fluvial processing (41). Within the Sheepbed member, the upper part has lower Cr/Ni and Zn/Ni , related mainly to higher Ni, consistent with a slightly different but still mafic provenance.

Lithium contents measured by ChemCam (42) also reveal a stratigraphic trend (Fig. 4C): Sheepbed mudstones have low and uniform Li abundances ($x = 4.3$ ppm; $\text{SD} = 2.4$ ppm), whereas Li abundances in the overlying units are greater by a factor of ~2 and are much more variable. Although secondary aqueous processes such as hydrothermal activity can enrich Li (43), and even though evidence for centimeter-scale Li redistribution is observed in diagenetic raised ridges (see below), this range for average values probably cannot be distinguished from variations in basaltic provenance, given our limited understanding of Li distributions in the martian crust-mantle system.

Early Diagenetic Features

Efforts were made to characterize chemical and mineralogical controls on concretion formation within the Sheepbed member. Numerous ChemCam observations were directed at concretions, but no systematic differences were observed, limiting compositional differences between concretion and host sediment to less than ~10% for major elements. APXS analyses of drill fines from concretion + hollow nodule-rich (Cumberland) and concretion + hollow nodule-poor (John_Klein) areas were also examined (25) (table S5). Samples delivered to the internal instruments (CheMin and SAM) that determined mineralogy (3, 4) are preferable and can be analyzed by APXS after being dumped onto the surface when CheMin and SAM analyses are complete (as for John_Klein). However, at the time of writing, Curiosity was still carrying the Cumberland sample, and consequently it has not been analyzed by APXS; instead APXS analyzed the fines that accumulated around the drill hole from the drilling process. Additionally, imaging of drill hole walls indicates that John_Klein has greater amounts of Ca sulfate-filled fractures (see below). A two-stage calculation was thus performed to evaluate results. First, 5% anhydrite was removed from the John_Klein composition to put SO_3 at similar concentrations in both samples, and to be broadly consistent with the relative amounts of Ca sulfate obtained from maps of veins in drill hole walls (4). Second, two gain-loss calculations were performed assuming that Ti and Al, respectively, are constant between samples. Elements enriched (taken as $\geq 5\%$ in both calculations) in Cumberland include Fe, Ca, Cl, Br, Ni, and Ge. Elevated Ca is difficult to interpret given the Ca sulfate in fractures, but elevated Fe, Cl, Br, and Ni are consistent with small amounts of a minor mineral such as akaganeite, identified by XRD, forming at least part of the concretion cement (4).

ChemCam and APXS analyses of isopachous cements within early diagenetic raised ridges indicate the presence of a Mg-Fe-Cl-rich phase (or assemblage). ChemCam confirms that the amount of MgO is as high as ~17%, and in places is accompanied by elevated Li (Fig. 5A). The observation that Li and Mg are not well correlated across the different layers of isopachous cements (e.g., right side of image in Fig. 5A) suggests a

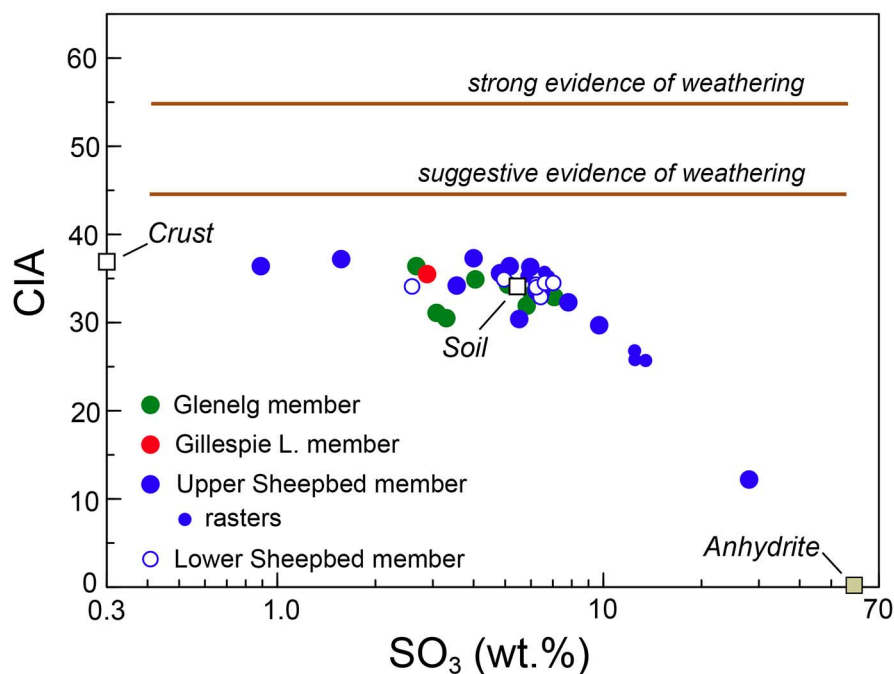


Fig. 3. Plot of CIA versus SO_3 contents for Yellowknife Bay formation APXS analyses. Shown for reference, as open squares, are average martian crust (12), local soil (64), the composition of anhydrite (CaSO_4) and horizontal lines that show the CIA values expected for basaltic sedimentary rocks that have experienced a chemical weathering history.

complex origin. APXS rasters on the raised-ridge target McGrath further indicates that Fe is elevated and that both Mg and Fe correlate with Cl, although enrichment of any chloride phase or oxychlorine compound explains only a tiny part of the Mg-Fe variation (Fig. 5B). A mass balance calculation, using the highest and lowest APXS MgO analyses from the McGrath raster (McGrath-R1 and -R2) and assuming that the component is ~20% of the rock, indicates a composition of ~45% SiO₂, ~35% FeO_T, ~18% MgO, ~3% Cl, and ~1300 to 1500 ppm each for Ni, Zn, and Br (25) (table S6). Such a composition cannot be accommodated by any single phase identified in the drilled samples by XRD, but perhaps is consistent with a mixture of Mg-rich, Al-deficient smectitic clay (e.g., hectorite, stevensite) and halogen-bearing Fe oxides (e.g., akaganeite).

Late Diagenetic Features

Both ChemCam and APXS provide constraints on the mineralogy of cross-cutting late diagenetic light-toned fractures, including filled hollow nodules. ChemCam shots on these features (Fig. 5C) show elevated Ca, S, and in places H, indicating multiple hydration states of Ca sulfate. ChemCam also measured elevated Sr content (up to 450 ppm) in the fracture fills (Fig. 5C), as expected for Ca sulfate (44). The presence of multiple Ca sulfate minerals is also consistent with CheMin XRD analyses that identified anhydrite and bassanite (but not gypsum) and MastCam visible-near infrared (VNIR) spectroscopy that suggests that some fracture fills are hydrated, possibly indicating gypsum (4). Finally, APXS raster analysis on the fracture fill target Sayunei also indicates the presence of Ca sulfates through a correlation between CaO and SO₃, the slope of which is consistent with CaSO₄ stoichiometry (Fig. 5D).

The composition of the dike-like “snake” feature (Snake_River target) may bear on its origin. The Snake_River major element composition is most similar to sedimentary rocks in the lower part of the Yellowknife Bay formation and best matches the lower Sheepbed member for major elements (Fig. 6A); it differs from the Gillespie Lake member for S, Cl, and all trace elements (Fig. 6B). Although close similarity exists with individual analyses of the heterogeneous Glenelg member, the “snake” does not compare favorably to the Glenelg average for a number of elements (Fig. 6C), which suggests that it probably did not result from infall of overlying sediment into a fracture. These comparisons indicate that the composition of the snake is consistent with a sedimentary dike—as suggested from stratigraphic relationships (2)—that intruded from the Sheepbed or other sediments at lower stratigraphic levels and that are not currently exposed. In detail, its trace element composition differs from all exposed units (higher Cr, lower Ni) and suggests an origin from a different, presumably lower, stratigraphic level.

Discussion

Elemental geochemistry reveals a fundamental provenance change during deposition of the Yellowknife Bay formation. Clastic sediments of the Sheepbed and Gillespie Lake members were derived from a source similar to the average martian crust but slightly more mafic and Fe-rich (SiO₂ ~ 46% versus 49%; FeO_T ~ 21.5% versus 18%), whereas high-K alkaline igneous rocks contribute substantially to the Glenelg member provenance. The change in provenance may be related to erosional evolution of drainage basins feeding the alluvial fan system. Down-cutting fluvial channels in the catchment of the fan system may have encountered a distinct alkaline basalt bedrock lithology (likely related to Jake_Matijevic-type composition) or captured a drainage underlain by such lithologies leading to the abrupt change in sediment provenance. On the other hand, alkaline basalts of the type

suggested to be incorporated into Glenelg sediments are rare on Earth (45); having them represent a dominant provenance component in distal facies of a fluvial system is unexpected and could suggest that such rocks, which have also been observed in Gusev crater (46), are more common on Mars than previously thought (45–47). An alternative possibility is that an exotic source of alkaline basalts was locally introduced into the basin, by way of volcanic ash or flows, and represents relatively small volumes of alkaline basalt that in turn were locally recycled and preserved as volcanoclastic layers. This scenario is consistent with the observation that high-K signatures appear restricted to certain beds within the Glenelg member. Given the limited stratigraphic distribution that has been studied (the Glenelg member exhibiting the high-K signature represents only ~1.7 m of a ~5-m section), it is not possible to distinguish whether relatively local

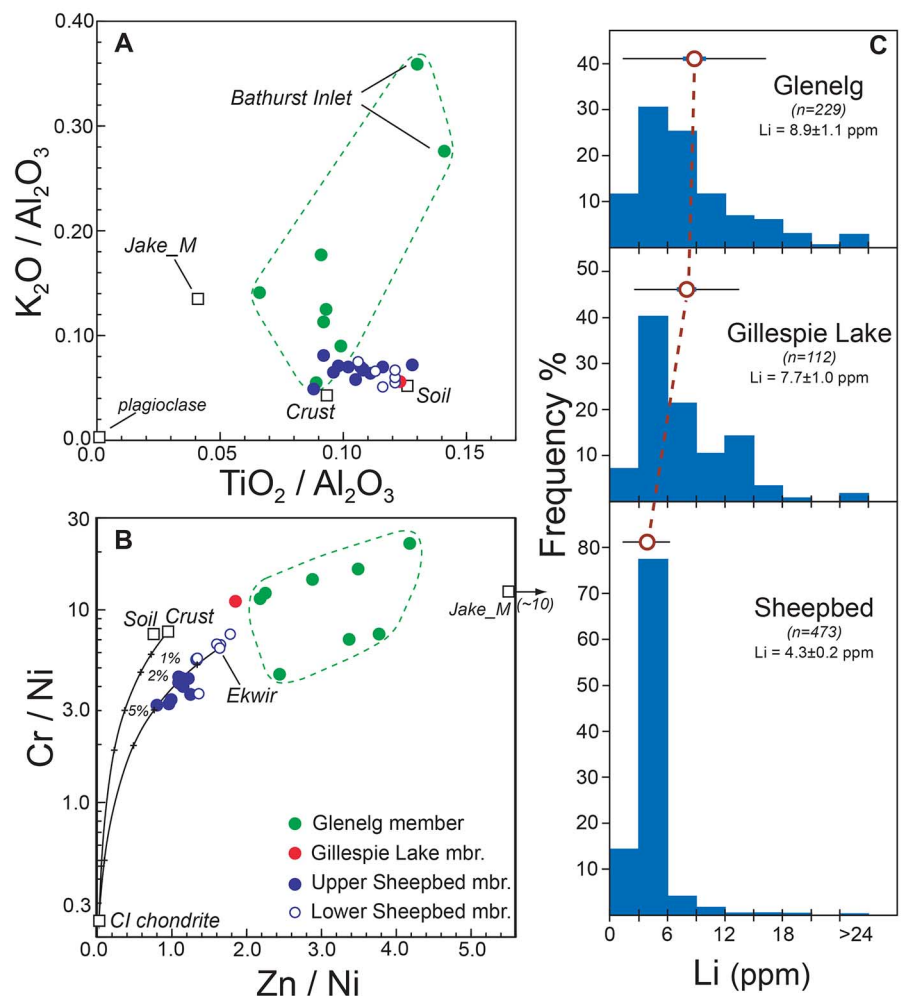


Fig. 4. Geochemical relationships within the Yellowknife Bay formation highlighting stratigraphic variations. (A) APXS K₂O/Al₂O₃ versus TiO₂/Al₂O₃; (B) APXS Cr/Ni versus Zn/Ni; (C) histograms of ChemCam Li abundances also showing mean (red circle), standard deviation (black bar) and 95% confidence interval (blue bar; also the uncertainty reported on the averages). The compositions of average martian crust (12), local soil (64), the rock Jake_Matijevic (45), average Cl chondrite (12) and plagioclase are shown for reference. Also shown in (B) are mixing lines between average martian crust and the Ekwir_Brush target and average Cl chondrite.

or more regionally exposed high-K lithologies caused the provenance change, and thus the scale of any potential alkaline igneous province is not well constrained from the sedimentary data.

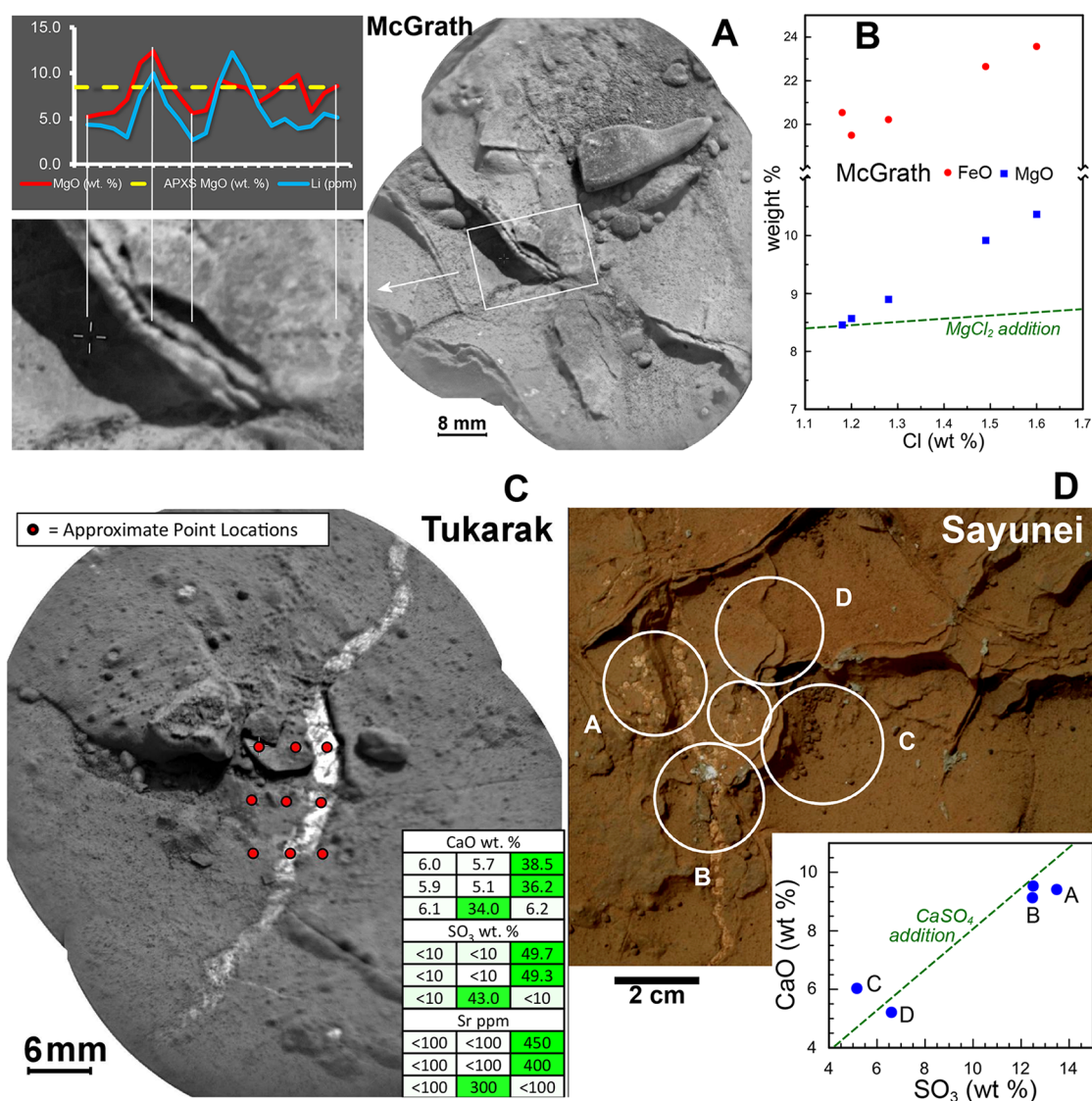
Martian soils likely contain a small (~1 to 3%) meteoritic component (48), and estimates of crustal composition (notably Ni) are derived by assuming a 2% meteoritic soil component (12). Sheepbed mudstones, if deposited in an ancient lake that represented the terminus of a watershed, might be expected to contain meteoritic material that was swept into the basin. Provenance effects alone can explain the levels of Ni (~450 to 850 ppm) in the Sheepbed member, given the slightly more mafic and Fe-rich composition relative to average martian crust. However, trace element data are also consistent with a modest meteoritic contribution. Modeling the effects of adding an average CI-type carbonaceous chondrite composition to average martian crust and to one of the brushed lower Sheepbed

analyses (Ekwir Brush) shows that the low Cr/Ni and Zn/Ni ratios of the Sheepbed member are consistent with ~1 to 4% CI component, with a larger amount in the upper part of the member (Fig. 4B). For typical CI-type chondritic compositions (49), a 1 to 4% meteoritic component could also deliver as much as ~300 to 1200 ppm of organic C, consistent with low levels of carbon detected by SAM (3).

Sediment chemistry, by constraining chemical weathering intensity, is one of the few tools available to evaluate paleoclimate in the absence of a fossil record (10, 50, 51). Low CIA values and positions on A-CN-K and A-CN-K-FM diagrams for the Yellowknife Bay formation (Fig. 2) indicate very limited chemical weathering before deposition. The similarity between major elements of the Gillespie Lake bedload-dominated sandstones and Sheepbed member suspended load-dominated mudstones also suggests that the fluvial system carried a very high ratio of solid

to dissolved river loads (L_s/L_d), which in turn results from both arid conditions and rapid erosion and transport (51, 52) and could have been further enhanced by cold conditions that would kinetically inhibit chemical weathering reactions. Accordingly, Yellowknife Bay formation geochemistry is consistent with some combination of a highly arid, possibly frigid, climate and/or a high-relief fluvial system, and probably both. On early Mars, impact processes may have aided in the generation of sedimentary particles, increasing the efficiency of physical denudation (53, 54). Surface waters with low L_d would be substantially different from the ancient surface waters of high ionic strength suggested at Meridiani Planum (55), even though both apparently record evidence of an arid climate system. Despite the suggested arid conditions, relatively small amounts of chemical sedimentation (e.g., sulfates, carbonates, chlorides) may be expected in the Yellowknife Bay sedimentary system. In addition, to the degree

Fig. 5. Geochemical constraints on diagenetic features within the Yellowknife Bay formation. (A) ChemCam RMI image of the McGrath target “raised ridge” that is dipping gently to the upper right. The inset shows LIBS MgO and Li transects for 20 shots taken across the feature. Shown for reference is the average MgO of McGrath, determined by APXS. Note elevated and correlated Mg and Li at the site of the raised ridge. Also note that elevated Mg (but not Li) is observed on the right side that likely represents the outer layer of the cement, exposed on the dipping surface. **(B)** APXS raster analysis for McGrath showing elevated Fe and Mg that correlate with Cl. Note the break in the scale on the y axis. A model of $MgCl_2$ addition is shown to illustrate that the correlation is not due simply to the presence of chloride phases. **(C)** ChemCam RMI image of a late diagenetic light-toned fracture at the Tukarak target. Location of a 3×3 LIBS raster is shown; results for Ca, S and Sr, relative to their position in the raster, are given in the inset table. **(D)** APXS raster analysis on late diagenetic light-toned fracture at the Sayunei target. The inset shows the plot of CaO versus SO_3 , with a model illustrating the effects of $CaSO_4$ addition.



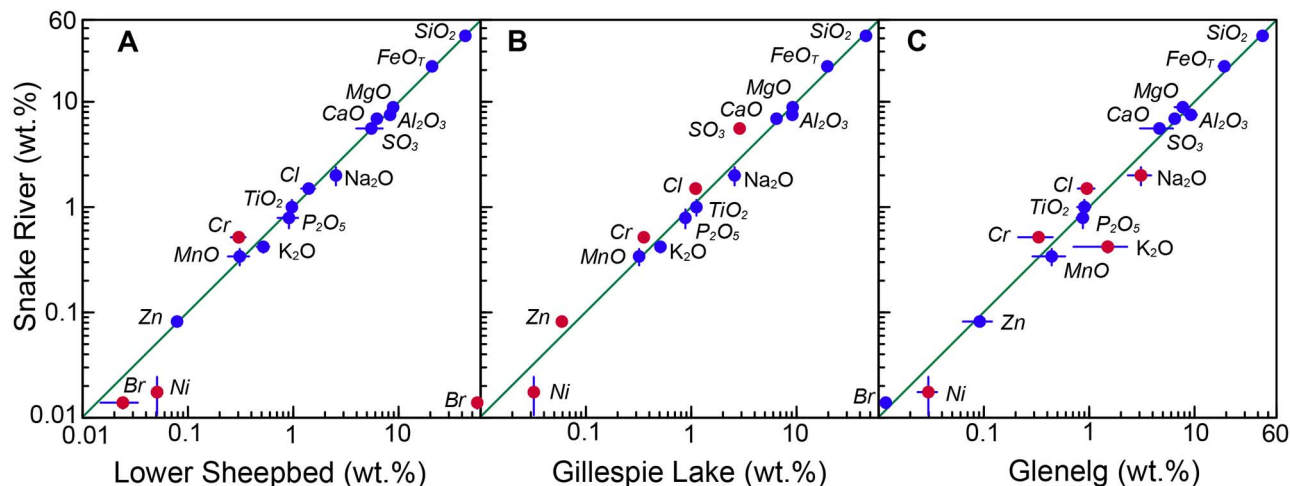


Fig. 6. Comparison diagrams for the dike-like feature, termed the “snake.” (A to C) APXS composition of the Snake River target compared to (A) lower Sheepbed member average, (B) Gillespie Lake member sample, and (C) Glenelg member average. The upper Sheepbed is not used for this comparison because relative to the lower Sheepbed, its geochemistry is more heavily influenced by

diagenetic features (e.g., Ca sulfate veins, concretions). The diagonal green line represents equal compositions. Error bars (if larger than the symbol size) represent 2σ errors for individual samples (Snake River, Gillespie Lake) and one standard deviation on the mean for averages (Lower Sheepbed member, Glenelg member). Significant differences in composition are shown as red symbols.

that this surface water contributes to the regional groundwater system, it would also tend to promote dilute, circumneutral pH, subsurface aqueous conditions.

Elemental geochemistry also provides constraints on diagenetic history. The uniform bulk rock compositions of Sheepbed mudstones are particularly important in this respect. Sheepbed’s suite of complex diagenetic features suggests that postdepositional aqueous alteration took place at combined low water/rock ratios and pH levels modest enough that mineralogical changes took place under nearly isochemical conditions; very little mass has been removed from the system. Therefore, the broad array of secondary minerals identified by XRD—including saponitic phyllosilicates, magnetite, akaganeite, hematite, and perhaps a substantial part of the amorphous component—needs to be explained by plausible reactions taking place within the sediment (4).

One important exception is that Ca sulfates found in fractures, voids, and hollow nodules were later “added” to the rock, as inferred from textural and geochemical relationships; that is, these Ca sulfates were not formed by local redistribution of elements during the earlier stage of diagenesis that transformed the Sheepbed sediment to rock. Thus, at least two distinct diagenetic fluid events took place with distinct fluid chemistry. The first fluid event (perhaps more than one such event given the mineralogical complexity, including coexistence of magnetite, akaganeite, pyrrhotite, and possibly hematite and pyrite) resulted in the mineral assemblage within the host sediment (and perhaps the Fe-Mg-Cl-rich assemblage associated with early diagenetic raised ridges). Plausible reactions that could be involved include olivine (+ Al) $\rightarrow$ saponite + magnetite (4) and pyrrhotite (+ pyrite?) $\rightarrow$ akaganeite ($\rightarrow$ hematite?), the latter of which would be promoted by Cl^- -

bearing fluids that also could have promoted concretion formation. Some time after lithification, an additional fluid event, presumably originating from deeper within the section and associated with fracturing of the mudstone, injected fluids that became saturated with respect to Ca sulfate (typically early-precipitated minerals in evolving brines) because of changing chemistry and/or pressure and temperature conditions. These fluids precipitated sulfates within the fractures and filled any void spaces that the fractures intersected, including the hollow nodules. Although considerable modeling and experimental effort will be required to fully understand these processes, it is possible to construct simple forward thermochemical aqueous models broadly consistent with these observations (25) (fig. S8).

Our findings add to the growing evidence for highly diverse sedimentary environments on early Mars (2, 56, 57). The Burns formation of Meridiani Planum is the only sedimentary sequence that has been studied in situ in comparable detail and is of similar age—if anything, even older (20, 57, 58). Although both represent clastic sedimentation derived from basalt sources and influenced by complex groundwater diagenesis, the sedimentary history is strikingly different. For example, where the Burns formation preserves evidence for groundwater of very low pH and very high ionic strength, the Yellowknife Bay formation had relatively dilute circumneutral groundwater. Where the Burns formation is dominated by chemically precipitated constituents (sulfates, chlorides) derived from evaporation of acidic brines, Yellowknife Bay mudstones contain very little in the way of chemically precipitated constituents and, apart from cross-cutting Ca sulfate veins, the secondary mineralogy formed mostly within a largely closed geochemical system. Whereas the basaltic debris within the Burns formation

was substantially chemically weathered before deposition, Yellowknife Bay detritus appears to be essentially unweathered. Orbital spectroscopic mapping suggests that surface aqueous environmental conditions on early Mars (late Noachian) evolved from circumneutral clay-rich to acidic sulfate-rich settings (59), analogous to the early Earth where evolving global surface environments are also reflected in the broad sweep of the geological record (60). However, on Mars, just as on Earth, geological complexities inevitably emerge when the sedimentary record is examined closely.

Materials and Methods

APXS is a well-established analytical technique on Mars, providing quantitative abundance data for major and minor elements, including S, Cl, and trace elements Cr, Ni, Zn, Ge, and Br (61). Analytical details are provided in (25); Yellowknife Bay formation results are provided in tables S1 to S4. Multiple APXS analyses were sometimes obtained on the same rock but at different locations. In some cases (e.g., Bathurst Inlet), both analyses are considered because they represent slightly different stratigraphic levels. However, where multiple analyses were made at a single site, such as drill sites (e.g., John Klein, Cumberland) and APXS “rasters” (e.g., Sayunei, McGrath), the analysis with lowest SO_3 was selected as most representative of the sedimentary rock because brushed surfaces indicate low SO_3 and because mapping of borehole walls suggests that Ca sulfate abundances correlate with diagenetic light-toned veins and hollow nodule fills. APXS “rasters” involving multiple closely spaced measurements were used on two diagenetic features: fracture fills (Sayunei) and raised ridges (McGrath). These results are provided in table S4, which also includes the suggested sedimentary dike (Snake River analysis). The ChemCam LIBS instrument provides

semiquantitative analyses for major and some minor and trace elements (e.g., Ba, Rb, Sr, Li) using multiple laser pulses (shots) on targets ~350 to 550 μm in diameter from distances of up to 7 m (62, 63). The first ~5 shots remove surface dust, and the rock analyses are based on averages of subsequent shots. Further analytical details are provided in (25).

References and Notes

- Names have been assigned to certain areographic features by the Mars Science Laboratory (MSL) team for planning and operations purposes. The names are not formally recognized by the International Astronomical Union.
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Acknowledgments: Much of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under contract with NASA. Development and operation of the ChemCam and APXS instruments were also supported by funds from the French Space Agency, CNES, and the Canadian Space Agency. Organizations supporting research include NASA, the Canadian Space Agency, NSERC

(Canada), and the United Kingdom Space Agency (UK). Chemical data presented here are derived from archived data sets in the NASA Planetary Data System (PDS), <http://pds-geosciences.wustl.edu/missions/msl>. We are grateful to the MSL engineering and management teams for making the mission and this scientific investigation possible and to science team members who contributed to mission operations. S.M.M. thanks Lamont-Doherty Earth Observatory of Columbia University, and especially S. Hemming, for hospitality during a sabbatical when the manuscript was being prepared.

Supplementary Materials

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15 August 2013; accepted 12 November 2013
Published online 9 December 2013;
[10.1126/science.1244734](https://doi.org/10.1126/science.1244734)



**Mars' Surface Radiation Environment Measured with the Mars
Science Laboratory's Curiosity Rover**
Donald M. Hassler *et al.*
Science **343**, (2014);
DOI: 10.1126/science.1244797

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Mars' Surface Radiation Environment Measured with the Mars Science Laboratory's Curiosity Rover

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The Radiation Assessment Detector (RAD) on the Mars Science Laboratory's Curiosity rover began making detailed measurements of the cosmic ray and energetic particle radiation environment on the surface of Mars on 7 August 2012. We report and discuss measurements of the absorbed dose and dose equivalent from galactic cosmic rays and solar energetic particles on the martian surface for ~300 days of observations during the current solar maximum. These measurements provide insight into the radiation hazards associated with a human mission to the surface of Mars and provide an anchor point with which to model the subsurface radiation environment, with implications for microbial survival times of any possible extant or past life, as well as for the preservation of potential organic biosignatures of the ancient martian environment.

The radiation exposure on the surface of Mars is much harsher than that on the surface of the Earth for two reasons: Mars lacks a global magnetic field to deflect energetic charged particles (*1*), and the martian atmosphere is much thinner (<1%) than that of Earth, providing little shielding against the high-energy particles that are incident at the top of its atmosphere. This environmental factor, for which there is no analog on Earth, poses a challenge for future human exploration of Mars (*2–9*) and is also important in understanding both geological and potential biological evolution on Mars. The radiation environment on Mars has been previously estimated and modeled (*10–17*). Here, we report in situ measurements of the ionizing radiation environment on the surface of Mars; these can be used to test and validate radiation transport models.

There are two types of energetic particle radiation incident at the top of the Mars atmosphere, galactic cosmic rays (GCRs) and solar energetic particles (SEPs). Both GCRs and SEPs interact with the atmosphere and, if energetic

enough, penetrate into the martian soil, or regolith, where they produce secondary particles (including neutrons and γ -rays) that contribute to the complex radiation environment on the martian surface, which is quite unlike that observed at the Earth's surface.

GCRs are high-energy particles [10 mega-electron volt per nuclear particle (MeV/nuc) to >10 GeV/nuc], fluxes of which are modulated by the heliosphere and anticorrelated with solar activity (*18*). The composition varies slightly depending on solar modulation, with the proton abundance in the range of 85 to 90%, helium ions ~10 to 13%, electrons ~1%, and ~1% heavier nuclei (*19, 20*). Because of their high energies, GCRs are difficult to shield against and can penetrate up to several meters into the martian regolith. SEPs are produced in the solar corona as a result of high-energy processes associated with flares, coronal mass ejections (CMEs), and their corresponding shocks. SEP events are sporadic and difficult to predict, with onset times on the order of minutes to hours and durations of hours to days. SEP fluxes can vary by several orders of magnitude and are typically dominated by protons, but composition can vary substantially (*21*). SEP protons and helium ions with energies below ~150 MeV/nuc ("soft" spectrum events) do not penetrate to the martian surface. Typical column depths of the martian atmosphere at Gale crater are on the order of 20 g/cm², thus energetic particles with energies less than ~150 MeV lose all of their energy before passing through this amount of material. However, during "hard spectrum" events ions can be accelerated to energies well above 150 MeV/nuc, with substantial fluxes reaching the martian surface. In all events, secondary neutrons produced by SEPs in

the atmosphere can reach the surface. The Radiation Assessment Detector (RAD) measurements reported here cover observations of GCRs as well as hard and soft SEP events seen from the martian surface. Together with the radiation environment results from RAD inside the Mars Science Laboratory (MSL) spacecraft during its cruise to Mars (*22*), these measurements correspond to all three phases (outbound interplanetary journey, Mars surface stay, and return journey) of a human Mars mission at this time in the solar cycle and thus are directly relevant to planning for future human missions.

If martian life exists, or existed in the past, it is reasonable to assume it is or was based on organic molecules (*23, 24*) and will therefore share with terrestrial life the vulnerability to energetic particle radiation (*25, 26*). Thus, we present here extrapolations of the RAD surface dose measurements (using transport models) to the martian subsurface, with implications for estimating lethal depths and microbial survival times (*26–30*). The radiation environment on Mars may also play a key role in the chemical alteration of the regolith and martian rocks over geologic time scales, affecting the preservation of organics, including potential organic biosignatures of the ancient martian environment (*26, 27*). The RAD surface measurements provide a baseline for inferring the flux in these more shielded environments (by validating and anchoring transport models) and thus the foundation for understanding the limits to preservation of organic matter in the soil and rocks of Gale crater.

Results and Discussion

The Curiosity rover landed successfully on Mars in Gale crater at ~4.4 km MOLA (Mars Orbiter Laser Altimeter) altitude on 6 August 2012. On 7 August 2012, the RAD began taking observations of the radiation environment on Mars, incidentally 100 years to the day after the discovery of cosmic rays on Earth by Victor Hess from a balloon in Austria (*31*). The results reported here are time series of absorbed dose rate, the average absorbed dose rate and average dose equivalent rate, and Linear Energy Transfer (LET) spectra for ~300 sols (1 martian sol = 24 hours 39 min.) from 7 August 2012 to 1 June 2013.

The radiation dose rate measured by RAD on the Mars surface during the first 300 sols on Mars is shown in Fig. 1, near the maximum of solar cycle 24. The GCR dose rate can be seen to vary between 180 and 225 microgray (μ Gy)/day, owing to the combined effects of diurnal variations from atmospheric pressure changes, Mars seasonal variations at Gale crater, and heliospheric structure variability due to solar activity and rotation.

The diurnal dose rates vary by a few percent because of diurnal change in the Mars atmospheric column, as can be seen in Fig. 2A, which shows data obtained between sols 290 and 302. This

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diurnal variation of the total atmospheric column mass is related to the daily thermal tides that Mars experiences each sol, by which the direct heating of the martian atmosphere by the Sun produces global-scale waves that redistribute atmospheric mass (33). Comparison of the RAD dose rate to the Rover Environment Monitoring Station (REMS) (34) atmospheric pressure measurements shows there is an anticorrelation between total dose rate and atmospheric pressure (Fig. 2B), which in turn is directly related to column depth.

On the Mars surface, during the 300-day period near the maximum of solar cycle 24, we found an average total GCR dose rate at Gale crater (-4.4 km MOLA) of 0.210 ± 0.040 mGy/day, compared with 0.48 ± 0.08 mGy/day measured during cruise inside the MSL spacecraft (Fig. 3 and Table 1). The difference in dose rate is driven by several influences: First, the shielding of the lower hemisphere provided by the planet reduces the dose rate by a factor of ~ 2 . Second, further deviations from this factor of two are due to interactions of primary GCRs with the nucleons in the atmosphere (and soil). Additionally, the effective atmospheric shielding is thicker than the spacecraft shielding of the instrument during cruise. The dose rate is also influenced by the modulation of the GCR flux by the sun—a stronger solar modulation results in overall lower GCR fluxes and thus lower dose rates. The solar modulation parameter during the surface mission to date has been ~ 577 MV, whereas the average Φ during cruise was ~ 635 MV (resulting in lower effective GCR flux).

We find the average quality factor $\langle Q \rangle$ on the martian surface to be 3.05 ± 0.3 , compared with 3.82 ± 0.3 measured during cruise. This smaller $\langle Q \rangle$ is due to the thicker shielding in the field of view (FOV) on the surface because during cruise, approximately half of the RAD FOV was lightly shielded (< 10 g/cm²) (35). The column depth of the martian atmosphere averaged about 21 g/cm² over the first 300 sols of Curiosity's mission. Combining the tissue dose rate measurement with $\langle Q \rangle$ yields an average GCR dose-equivalent rate on the Mars surface of 0.64 ± 0.12 millisieverts (mSv)/day (Fig. 4).

The SEP dose was obtained by subtracting the average GCR dose rate for the duration of the SEP event. It was found to be 50 μ Gy in the less-shielded of the two detectors used for dosimetry. Because the composition of SEP events (observed both on the surface and during cruise) are dominantly protons, for which $\langle Q \rangle = \sim 1$, the dose equivalent from this event was ~ 50 μ Sv, approximately equal to 25% of the GCR dose equivalent for the 1-day duration of the event.

The frequency and intensity of SEP events is highly variable and still unpredictable, and although these observations were made near solar maximum, this current solar activity cycle is very weak by historical norms (36). Substantial SEP events throughout recent history (such as February 1956, August 1972, and September 1989) have been reported and modeled to be several orders

of magnitude more intense than those currently observed to date by the RAD (37).

Implications for Future Human Missions to Mars

Combining our measurements with those obtained during the cruise phase (22), we estimate a total mission dose equivalent of ~ 1.01 Sv for a round trip Mars surface mission with 180 days (each way) cruise, and 500 days on the martian surface for this current solar cycle (Table 2). These mission phase durations are based on one possible NASA design reference mission (38); many mission designs and many mission windows at

different times in the solar cycle or a different solar cycle would result in somewhat different radiation exposures. Because GCR flux is modulated by solar activity (decreasing during solar activity maximum and increasing during solar activity minimum) and the risk for exposure to SEPs increases with solar activity, the contribution of each to the total mission dose of a future Mars mission depends on when in the solar cycle the mission occurs (3–6).

Estimates of Subsurface Dose Rates

The dose and dose-equivalent rates reported in Tables 1 and 2 can be extrapolated to obtain rates

Fig. 1. Time series of radiation dose rate measured by RAD on the surface of Mars.

During this time, RAD observed a dose rate enhancement from one hard SEP event on sol 242 (12 to 13 April 2013) and several Forbush decreases (32), resulting from soft SEP event-related interplanetary coronal mass ejections (ICMEs) on sols 50, 97, 208, and 259. (These ICMEs serve as magnetic shields against the GCR, thus reducing the observed flux.) Occasional brief gaps can also be seen, usually caused by RAD having been powered off so that other activities could take place on the spacecraft without interference.

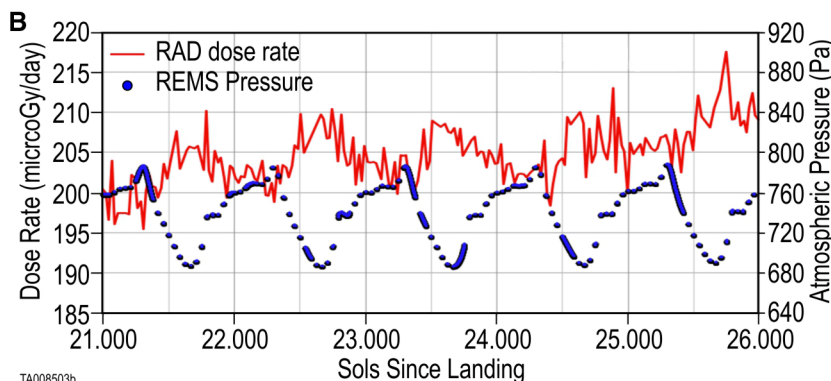
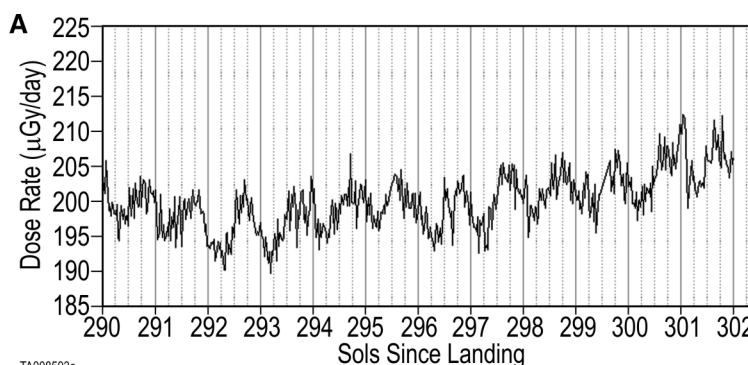
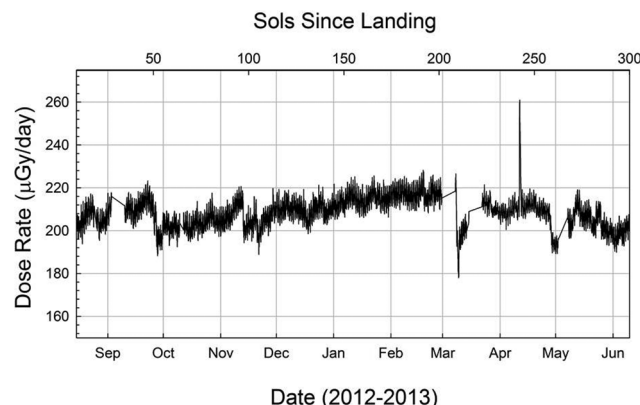


Fig. 2. Comparison of RAD dose rate versus time and atmospheric pressure. (A) RAD daily dose rate versus time. **(B)** Comparison of RAD dose rate with REMS atmospheric pressure.

below the martian surface by using the surface measurements to anchor model predictions. Refining estimates of the subsurface radiation environment is important because in situ regolith-based materials are prime candidates for astronaut shelter shielding materials in order to reduce or mitigate the biological hazards associated with radiation exposures on future long-duration human missions. These improved subsurface radiation estimates give insight into the potential for the preservation of possible organic biosignatures as a function of depth as well as survival times of possible microbial or bacterial life forms left dormant beneath the surface.

Several studies have modeled the expected subsurface radiation regime (26, 39), but the dose values depended until now on the modeled radiation environment on the surface. Dartnell *et al.* (26, 27) assumed an absorbed dose of ~ 150 mGy/year at the martian surface, whereas Pavlov *et al.* (28, 29) assumed an absorbed dose of 50 ± 5 mGy/year. The actual absorbed dose measured by the RAD (76 mGy/year at the surface) (Table 3) allows for more precise estimations of the subsurface dose. Differences may be in part due to differing assumptions in the models about the level of solar modulation compared with the

actual level during the measurement period as well as the amount of atmospheric shielding above the surface. Also, all of the above models must assume a rock, ice, or soil density. On the basis of compositional and morphological observations of the rocks at the John Klein site in Gale crater (42), we estimate a rock density of 2.8 g/cm^3 , which approximates the density of an iron-rich mudstone or siltstone. Although our estimates of subsurface dose depend strongly on the models we used, they are useful for comparison purposes. The natural background radioactivity on present-day Mars is thought to be on the order of $\sim 1 \text{ } \mu\text{Gy/day}$ (43), suggesting that GCR radiation is no longer the dominant source of radiation below $\sim 3 \text{ m}$. This also implies that the effectiveness of regolith-based shielding materials no longer improves beyond a thickness of $\sim 3 \text{ m}$.

Implications for Microbial Survival Times

Energetic particles ionize molecules along their tracks. The energy deposited by ionization or excitation greatly exceeds that required to break many molecular bonds—including those in DNA, other organic molecules, and water—thus, ionizing radiation is extremely damaging to biomolecules through both direct and indirect mechanisms. Thus, measurements of the surface and subsurface radiation environment are critical for estimating the survival probability and survival times of possible dormant life forms found in the martian soil, regolith, rock, and ice. For this, the dose rates can be used to calculate the time it would take for different bacterial species to accumulate a lethal dose of radiation in different subsurface depths (44).

Even the radioresistant organism *D. radiodurans* would, if dormant, be eradicated in the top several meters in a time span of a few million years (28, 29). However, inferred recurring climate changes in the post-Noachian era, due to variations in the planetary obliquity on time scales of several hundred thousand to a few million years (45), could lead to recurring periods of metabolic activity of these otherwise dormant life forms. In this case, it is hypothesized that accumulated radiation damages could be repaired, and the “survival clock” of such life forms could be reset to zero for the next dormant phase (26, 28), which could in turn lead to possible survival to present times. It has been (27) estimated that a 2-m-depth drill was necessary to access viable radioresistant cells that may have gone through this reanimation step within 450,000 years. Applying the RAD dose results, we estimate that only a 1-m-depth drill is necessary to access the same viable radioresistant cells.

Implications for the Preservation of Environmental Records and Organic Biosignatures

Whether the bulk of the martian atmosphere was lost before the Noachian era (~ 3.7 to 4.0 billion years ago), as recent isotope ratio measurements by Curiosity suggest (46), or toward the

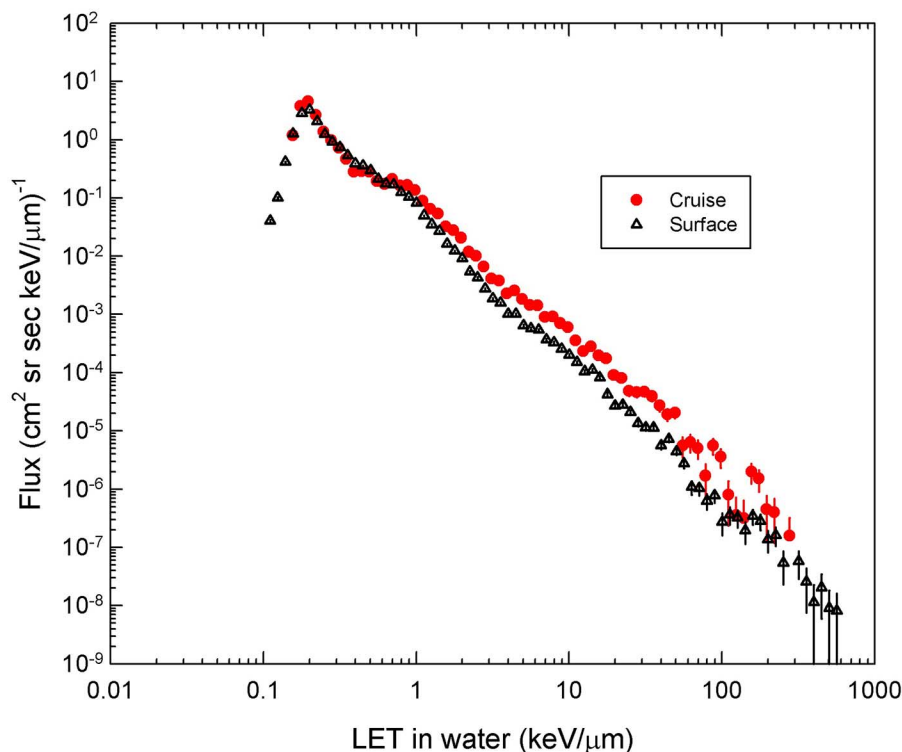


Fig. 3. Charged-particle LET spectrum comparison. Shown is a comparison of charged-particle LET spectrum measured on the Mars surface (black) with that measured during cruise inside the MSL spacecraft (red) with variable shielding (22). The energy deposited in silicon has been converted to LET in water.

Table 1. Radiation environment measured by MSL/RAD (2012–2013) (GCR only). Charged-particle fluxes for both cruise and surface were calculated by using the single-ended geometric factor for a two-detector coincidence ($0.90 \text{ cm}^2 \text{ sr}$). Fluence rates were calculated by using all hits above threshold in a single detector (B, with area 1.92 cm^2). Solar modulation was, on average, slightly stronger during the first 300 sols on the surface than during cruise.

RAD measurement	Mars surface	MSL cruise	Units
Charged-particle flux (A * B)	0.64 ± 0.06	1.43 ± 0.03	$\text{cm}^2/\text{s}/\text{sr}$
Fluence rate (B)	1.84 ± 0.34	3.87 ± 0.34	cm^2/s
Dose rate (tissue-like) (E detector)	0.21 ± 0.04	0.48 ± 0.08	mGy/day
Average Quality Factor <Q>	3.05 ± 0.26	3.82 ± 0.30	(dimensionless)
Dose-equivalent rate	0.64 ± 0.12	1.84 ± 0.30	mSv/day
Total mission dose equivalent [NASA design reference mission]	320 ± 50 (500 days)	662 ± 108 (2×180 days)	mSv

end of the Noachian era (39, 47–49), it is thought that the martian surface has had little protection from energetic particles for most of its history (50). Over such geologic time scales, an enormous fluence of high-energy charged particles (both primary and secondary) has interacted with, and most likely altered, the martian regolith, contributing substantially to the distinct chemistry of the martian soil and rocks (51, 52) and affecting the preservation of environmental records. The assessments of habitability and potential biosignatures of any ancient environment depend on the robustness of the preserved record, and ionizing radiation strongly influences chemical

compositions and structures, especially for water, salts, and redox-sensitive components such as organic matter (53–56). Carbon isotopic compositions may also be altered in the upper 50 cm of rock and soil (28). Organic molecules hold high potential for recording biosignatures (57), and organic matter (biogenic or abiogenic) may provide a source of carbon for habitable environments (42). Our RAD surface measurements and subsurface estimates constrain the preservation window for martian organic matter after exhumation and exposure to ionizing radiation in the top few meters of the martian surface. Prior studies focused on the top few centimeters

of rock, such as that accessible by the MSL drill. Using the amino acid degradation rates observed by (58), Pavlov *et al.* (29) modeled a ~99.9% decrease in 100–atomic mass unit molecules in ~1 billion years at 4- to 5-cm depth. The higher dose rate to rocks determined by RAD reduces this period to ~650 million years. They postulated that higher-mass molecules would degrade much faster, assuming a molecular chemistry comparable with that of amino acids. Although this assumption is suitable for biomolecules (proteins) of endolithic organisms, it is not representative of martian biomolecules that survive early diagenesis in sediments, geological organic matter in basalts (59), or exogenously delivered organics (60). Degradation rates for molecules of other organic chemistry are not reported, but survival of organic matter in carbonaceous chondrites demonstrates that meteoritic organic matter survives ionizing radiation for billions of years.

Regardless of the source of martian organic matter (meteoritic, geological, or biological), its bonds are susceptible to cleavage and radical formation by ionizing charged-particle radiation. Permanent bond scissions, subsequent cross-linking with other radicals, and volatile formation can occur. Radicals that are formed from cleaved bonds are highly reactive and will react with inorganic and organic chemicals in the immediate environment. In the presence of both radiation and reactive environmental chemicals, organic matter is highly susceptible to alteration and eventual destruction. Irradiation of water and hydroxyl (–OH) groups produces free radicals and molecules (H⁺, OH⁺, and H₂O₂) that will oxidize hydrocarbons and aromatic macromolecules to produce small organic salts and CO₂ via Fenton reactions (61). On Mars, this oxidation process is likely accelerated by the presence of iron mineral catalysts. Further, ionizing radiation plays a key role in the formation of oxychlorine compounds in the atmosphere (62) and ices (63), which have been deposited in sediments (64–66) where they may have undergone radiolysis (52), causing eventual oxidation of any organics by the resulting products.

Although the presence of martian organic matter has not been confirmed via in situ observation, our RAD measurements suggest that the most favorable conditions for finding evidence of organics on Mars is in rocks or soils that have been more recently exposed (such as eroded canyon walls or recent impact craters) and do not show signs of aqueous activity after exhumation.

Materials and Methods

The RAD instrument (67) consists of a combined charged and neutral particle detector, with a solid-state detector telescope, CsI calorimeter, and plastic scintillator for neutron detection. Active coincidence logic discriminates against charged particles entering the detector from outside the charged-particles telescope’s field

Fig. 4. Radiation dose-equivalent comparison. Shown is a comparison of the radiation dose equivalent for a 500-day surface stay to that from a 180-day transit to Mars (22), a 6-month stay on the International Space Station (ISS) at 420 km altitude during the 2013 solar maximum, and several earth-based sources of radiation. Dose is a purely physical quantity, with units of gray or milligray.

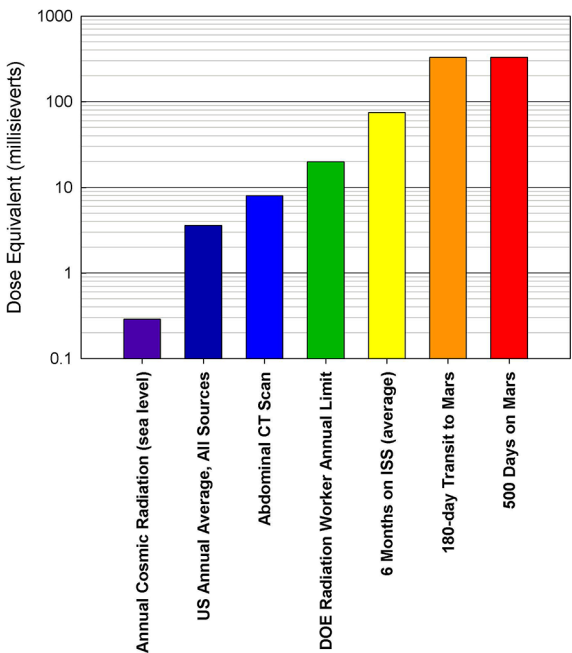


Table 2. Mars radiation environment summary during 2012–2013 solar maximum (GCR and SEP). The GCR dose rates are per day, and the SEP doses are per event, showing a range from the sampling of five (medium-size) SEP events observed during cruise and the one (small) event observed on the surface. Although the one SEP event observed on the martian surface was small, it is our only statistical sampling to date (materials and methods).

	GCR dose rate (mGy/day)	GCR dose-equivalent rate (mSv/day)	SEP dose (mGy/event)	SEP dose equivalent (mSv/event)
MSL Cruise (22)	0.464	1.84	1.2 to 19.5	1.2 to 19.5
Mars Surface	0.210	0.64	0.025	0.025

Table 3. Mars subsurface radiation estimates (scaled to RAD surface measurements). Both subsurface dose estimates and dose equivalent rated were determined by scaling HZETRN model (40, 41) calculations to RAD surface measurement values (Table 2).

Depth below surface	Effective shielding mass (g/cm ²)	GCR dose rate (mGy/year)	GCR dose-equivalent rate (mSv/year)
Mars surface (RAD)	0	76	232
–10 cm	28	96	295
–1 m	280	36.4	81
–2 m	560	8.7	15
–3 m	840	1.8	2.9

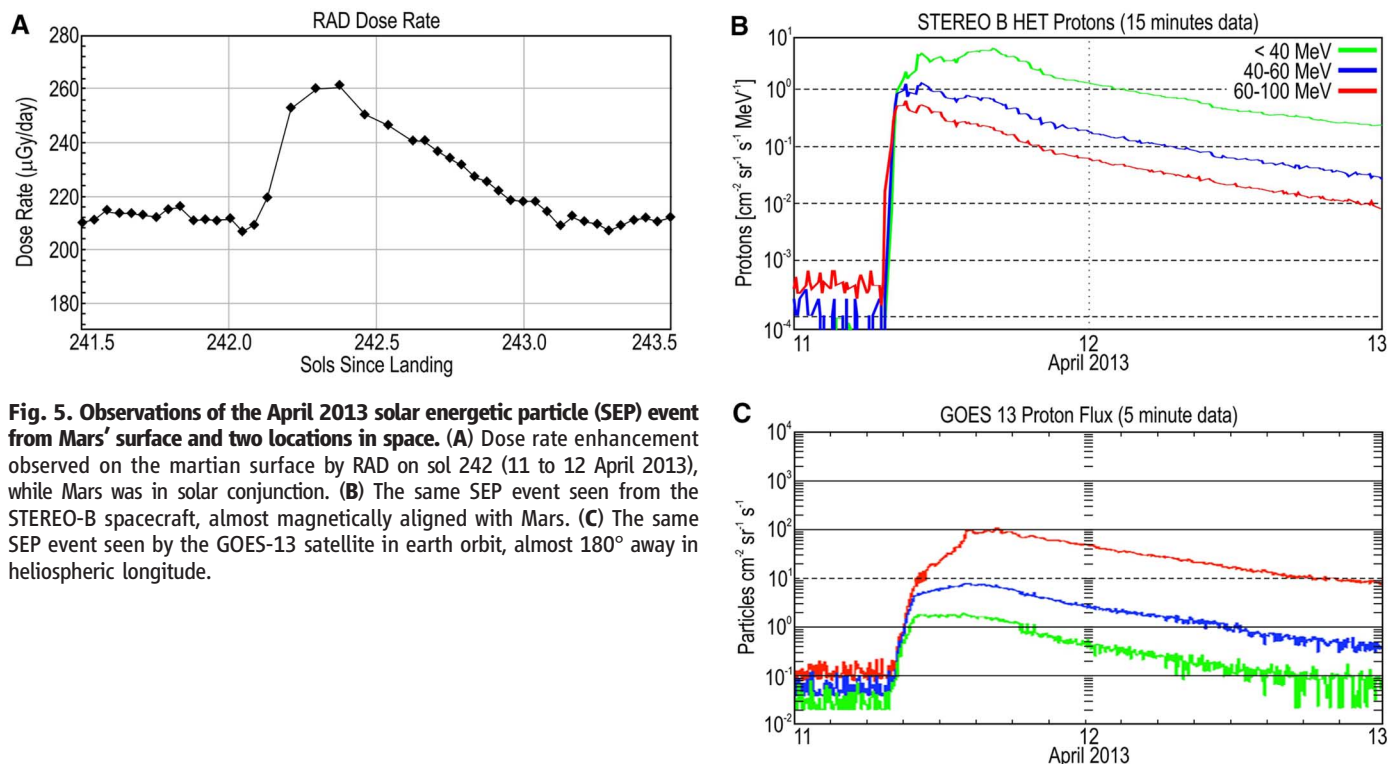


Fig. 5. Observations of the April 2013 solar energetic particle (SEP) event from Mars' surface and two locations in space. (A) Dose rate enhancement observed on the martian surface by RAD on sol 242 (11 to 12 April 2013), while Mars was in solar conjunction. **(B)** The same SEP event seen from the STEREO-B spacecraft, almost magnetically aligned with Mars. **(C)** The same SEP event seen by the GOES-13 satellite in earth orbit, almost 180° away in heliospheric longitude.

of view, and anti-coincidence logic enables detection of neutrons and γ -rays. The RAD has a wide dynamic range for charged particles and is able to measure all ion species that contribute to the radiation exposure on the surface of Mars with a geometry factor of $0.9 \text{ cm}^2 \text{ sr}$. The RAD measures differential fluxes of stopping charged particles with energies up to 95 MeV/nuc for protons and ^4He , and up to 450 MeV/nuc for ^{56}Fe . Neutral particles are identified in the energy range from $\sim 8 \text{ MeV}$ to 100 MeV. The dE/dx resolution of the RAD is sufficient to distinguish between major particle species. The RAD measures dE/dx in silicon, but these measurements can also be approximately related to LET in water. The RAD dynamic range corresponds to the LET range from 0.2 to $\sim 1000 \text{ keV}/\mu\text{m}$ in water.

Dose equivalent is determined by convoluting the LET spectrum of the measured particles with a quality factor, $Q(L)$ (68), that is an approximate measure of biological effectiveness of different radiation types. Dose is a purely physical quantity, with units of gray or milligray ($1 \text{ Gy} = 1 \text{ J/kg}$). Dose equivalent also has units of joules per kilogram but is expressed in sieverts or millisieverts.

Observations of SEP Event on 11 April 2013

The dose rate time series associated with the SEP event enhancement seen on 11 to 12 April 2013 resulting from an M-class flare on the Sun is shown in Fig. 5A. Although the SEP event appeared relatively weak in terms of flux increase as seen from Earth (GOES-13) (69), its energy spectrum was hard enough to produce an enhancement of $\sim 30\%$ over the GCR dose rate on

the martian surface. The 40- to 100-MeV proton flux seen by STEREO-B (70) increased almost four orders of magnitude at the peak of this event (Fig. 5B). The minimum proton energy required to reach the surface in Gale crater is $\sim 150 \text{ MeV}$. STEREO-B was leading Mars (in longitude) at the time of the event and had similar, but not identical, magnetic connection to the Sun. This event was the first “hard spectrum” SEP event seen by RAD on the Mars surface. Because Mars was in solar conjunction at this time, GOES-13 was nearly 180° in heliospheric longitude away, with fluxes of >50 and $>100 \text{ MeV}$ protons increasing by only two orders of magnitude (Fig. 5C). This SEP event was very broad in heliospheric extent, expanding to greater than 180° in heliographic longitude from the Sun. (This event was not observed by STEREO-A, which was trailing Mars at the time.) These observations from the RAD provide an additional data point to test models of the three-dimensional structure and propagation of SEPs through the inner heliosphere.

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Acknowledgments: This paper is dedicated to Dr. Michael J. Wargo at NASA HQ, who passed away unexpectedly on 4 August 2013. Mike was Chief Exploration Scientist in the Human Exploration and Operations Mission Directorate (HEOMD) and an enthusiastic supporter of collaborative projects between Science and Exploration. He was a strong supporter of RAD and a valuable member of both the science and exploration communities. He was a good friend and a wonderful human being, and he will be greatly missed. RAD is supported by NASA under Jet Propulsion Laboratory (JPL) subcontract 1273039 to Southwest Research Institute and in Germany by Deutsches Zentrum für Luft- und Raumfahrt (DLR) and DLR's Space Administration grant numbers 50QM0501 and 50 QM1201 to the Christian Albrechts University, Kiel. Part of this research was carried out at JPL, California Institute of Technology, under a contract with NASA. We extend sincere gratitude to J. Simmonds and J. Crisp at JPL; G. Allen, M. Meyer, C. Moore, V. Friedensen, and R. Williams at NASA HQ; and H. Witte at DLR in Germany for their unwavering support of RAD over the years. The authors also thank the reviewers for their careful and thoughtful comments and suggestions. The data used in this paper are archived in the NASA Planetary Data System's Planetary Plasma Interactions (PPI) node at the University of California, Los Angeles. The archival volume includes the full binary raw data files, detailed descriptions of the structures therein, and higher-level data products in human-readable form. The PPI node is hosted at <http://ppi.pds.nasa.gov>.

Supplementary Materials
www.sciencemag.org/content/343/6169/1244797/suppl/DC1
 Full Author List

16 August 2013; accepted 13 November 2013
 Published online 9 December 2013;
[10.1126/science.1244797](#)



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Science **343**, (2014);

DOI: 10.1126/science.1245267

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Volatile and Organic Compositions of Sedimentary Rocks in Yellowknife Bay, Gale Crater, Mars

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H₂O, CO₂, SO₂, O₂, H₂, H₂S, HCl, chlorinated hydrocarbons, NO, and other trace gases were evolved during pyrolysis of two mudstone samples acquired by the Curiosity rover at Yellowknife Bay within Gale crater, Mars. H₂O/OH-bearing phases included 2:1 phyllosilicate(s), bassanite, akaganeite, and amorphous materials. Thermal decomposition of carbonates and combustion of organic materials are candidate sources for the CO₂. Concurrent evolution of O₂ and chlorinated hydrocarbons suggests the presence of oxychlorine phase(s). Sulfides are likely sources for sulfur-bearing species. Higher abundances of chlorinated hydrocarbons in the mudstone compared with Rocknest windblown materials previously analyzed by Curiosity suggest that indigenous martian or meteoritic organic carbon sources may be preserved in the mudstone; however, the carbon source for the chlorinated hydrocarbons is not definitively of martian origin.

Curiosity landed in Gale crater on 6 August 2012 (UTC) with a goal to explore and quantitatively assess a site on Mars' surface as a potential habitat for past or present life. A topographic low informally named Yellowknife Bay located about 0.5 km northeast of the landing site was chosen as the first major exploration target because strata exposed were inferred to be fluvio-lacustrine deposits (*1*). Fluvio-lacustrine depositional systems are thought to preserve measurable evidence of paleo-habitability (*2*), e.g., factors such as mineral associations, elemental inventory, redox state, and character of light elements and compounds.

Curiosity entered Yellowknife Bay on sol 125 (12 December 2012) and began a drilling campaign to obtain powder samples from a mudstone located near the base of an exposed stratal succession (*3, 4*). Two drill samples informally named John Klein (drilled on sol 183) and Cumberland (drilled on sol 279) were extracted for delivery to the Sample Analysis at Mars (SAM) (*5*) and Chemistry and Mineralogy (CheMin) (*6*) instruments. The samples were obtained from the lowermost stratigraphic unit in the Yellowknife Bay formation, informally named the Sheepbed member (*1*). The Sheepbed member and an overlying medium- to coarse-grained sandstone, named the Gillespie Lake member, appear to have a complex postdepositional aqueous history. Apparent low matrix permeability in these units suggests that they were lithified during an early diagenetic event followed by at least one additional aqueous

episode when Ca-sulfate minerals precipitated in a network of intersecting fractures (*7*). Millimeter-sized nodules and hollow nodules in the Sheepbed member are interpreted, respectively, to be concretions and void spaces possibly formed as trapped gas bubbles during early diagenesis and lithification (*1*). The geology, stratigraphy, and diagenetic history of Yellowknife Bay are described in detail in companion papers (*1, 7, 8*).

The John Klein (JK) and Cumberland (CB) targets were drilled about 3 m apart in the Sheepbed mudstone and within ~10 cm of the same stratigraphic position. The JK drill hole intersected thin Ca-sulfate-rich veins. The CB sample was collected from an area rich in nodules and poor in Ca-sulfate-rich veins, to aid in mineralogical and geochemical characterization of the nodules. Powders extracted from both holes were gray in color, suggesting a relatively unoxidized material (*1, 8*), in contrast to the red-colored, oxidized materials observed earlier by Curiosity at the Rocknest aeolian deposit (*9, 10*) and other surface soils (*11*) encountered by previous missions (*12*). Additional details on the drill holes are described in (*1*) and (*8*), including maps of light-toned fractures in the drill hole walls (*8*).

Here we describe the volatile and organic C content of the Sheepbed mudstone and evaluate its potential for preservation of organic C. Volatile-bearing phases (including possible organic material) in Sheepbed are indicators of its past environmental and geochemical conditions and can shed light on whether the environment re-

corded in this mudstone once was habitable, i.e., met the requirements for microbial life as known on Earth (*13*). The volatile and organic compositions of JK and CB materials were characterized by the SAM instrument's evolved gas analysis (EGA), gas chromatography-mass spectrometry (GCMS), and tunable laser spectroscopy (TLS) experiments (*14*). Four JK subsamples (JK-1, JK-2, JK-3, and JK-4) and four CB subsamples (CB-1, CB-2, CB-3, and CB-5) of the <150- μ m-size fraction of drill fines were delivered to SAM for EGA and GCMS analyses (*15*).

Evolved H₂O

The most abundant gas evolved from JK and CB materials was H₂O. H₂O abundances released from JK [1.8 to 2.4 weight percent (wt %) H₂O] and CB (1.7 to 2.5 wt % H₂O; Table 1) were similar to those of Rocknest (1.6 to 2.4 wt % H₂O)

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(10). Other major evolved gases, in descending order of abundance, were H₂, CO₂, SO₂, and O₂ from JK and H₂, O₂, CO₂, and SO₂ from CB (Table 1 and Figs. 1 and 2).

An independent estimate of the volatile inventory of JK and CB can be obtained from measurements made by the Alpha Particle X-ray Spectrometer (APXS) (16). The measurement calculates the bulk concentration of the aggregate of excess light elements (including H₂O, CO₂, C, F, B₂O₃ and Li₂O) using the relative intensities of Compton- and Rayleigh-scattering peaks (14, 17). Estimates of the average excess light-element concentrations for the JK drill tailings (APXS measurement on sol 230) and the CB drill tailings (sol 287) were 4.3 (±5.5) and 6.9 (±6.2) wt %, respectively. The two methods for determining volatile abundances in the mudstone are consistent within uncertainties.

The JK and CB samples showed similar releases of H₂O in EGA experiments with a continuous temperature ramp (Figs. 1A and 2A) (18, 19). Evolved H₂O from JK (JK-4) resulted in two major H₂O releases, with very broad peaks at about 160° and 725°C (Fig. 1A). Cumberland samples exhibited similar behavior (Fig. 2A). The majority (~70%) of H₂O was driven off in the lower-temperature peak.

CheMin results constrain the potential phases releasing H₂O in the lower peak in JK and CB samples. CheMin detected basaltic silicate minerals (feldspar, pyroxene, olivine), magnetite (magnetite), anhydrite, bassanite, akaganeite, sulfides,

and ~30 wt % x-ray amorphous components in addition to a 2:1 trioctahedral phyllosilicate in JK and CB (8). Therefore, candidates for the lower-temperature water release are H₂O adsorbed on grain surfaces, interlayer H₂O associated with exchangeable cations in 2:1 phyllosilicates (e.g., smectite), structural H₂O (e.g., bassanite), structural OH (e.g., Fe-oxyhydroxides such as akaganeite), and occluded H₂O in glass or minerals. Adsorbed H₂O and interlayer H₂O in 2:1 phyllosilicates will generally release water below 300°C. Bassanite (CaSO₄·½H₂O) dehydrates at ~150°C. Akaganeite [FeO(OH,Cl)] undergoes dehydroxylation at ~250°C (fig. S2). H₂O incorporated into the amorphous components (e.g., nanophase Fe-oxides, allophane/hisingerite) may also evolve below 450°C. Water as liquid or vapor inclusions in glass or minerals would be released over a wide range of temperatures. Additional sources of evolved H₂O at low temperatures not constrained by CheMin include structural H₂O in oxychlorine compounds (e.g., hydrated perchlorates), structural OH in organics, and H₂O formed during organic reactions in the SAM pyrolysis oven. Organic matter can release H₂O over a wide range of temperatures from structural O and H as a consequence of reactions that take place in the SAM oven.

The high-temperature H₂O release between 450° and 835°C is consistent with the dehydroxylation of the octahedral layer of a 2:1 phyllosilicate (e.g., smectite), although other phases that contain OH in octahedral layers may also evolve H₂O in this temperature region. The basal (001)

spacing of the JK phyllosilicate measured by CheMin was mostly collapsed to ~10 Å suggesting a 2:1 phyllosilicate with little interlayer H₂O (8). The 2:1 phyllosilicate in the CB sample was expanded, with a basal (001) spacing of ~13 to 14 Å, consistent with several possible interpretations; the CB phyllosilicate could be smectite with an interlayer partially occupied by metal-hydroxyl groups or smectite with high-hydration-energy cations (e.g., Mg²⁺), facilitating retention of H₂O (8). The high-temperature H₂O releases at 750°C can be ascribed to dehydroxylation of Mg- and Al-enriched octahedral sheets in 2:1 phyllosilicates (Fig. 3A). The most likely candidate for the high-temperature H₂O release (i.e., ~750°C) in Sheepbed material is saponite or Fe-saponite based on CheMin measurement of the 02ℓ diffraction band (4.58 Å) as consistent with a trioctahedral 2:1 phyllosilicate (8) and the water-release peak temperature (Fig. 3A). If all of the H₂O released between 450° and 835°C during SAM pyrolysis runs resulted from dehydroxylation of 2:1 phyllosilicates, the proportions of 2:1 phyllosilicate present in the JK and CB samples are 17 (±12) wt % and 16 (±11) wt %, respectively. These values are consistent with the independent estimates from CheMin x-ray diffraction semiquantitative data, which give 22 (±11) and 18 (±9) wt % for 2:1 phyllosilicate in JK and CB, respectively (8).

High-temperature release of H₂ occurs over roughly the same temperature regions as the high-temperature releases of H₂O and H₂S (Figs. 1

Table 1. Evolved gas abundances released during SAM pyrolysis runs of samples (<150 μm fraction) obtained from the John Klein and Cumberland drill holes. Rocknest aeolian material evolved gas abundances are provided for comparison with the Sheepbed mudstone materials.

	John Klein				Cumberland			CB- 5	Rocknest† Average of four runs
	JK-1	JK-2	JK-3*	JK- 4	CB-1	CB-2	CB-3		
<i>Molar abundances (μmol)</i>									
H ₂ O	57.4 ± 32.7‡	54.2 ± 30.8	59.9 ± 33.7	44.7 ± 25.0	42.7 ± 28.8	54.0 ± 29.5	62.4 ± 31.5	45.0 ± 25.4	55.1 ± 35.3
CO ₂	7.0 ± 1.9	8.1 ± 1.7	6.6 ± 1.6	5.7 ± 1.3	2.0 ± 0.5	2.5 ± 0.7	3.1 ± 0.6	3.1 ± 0.7	9.9 ± 1.2
SO ₂	1.6 ± 0.6	1.4 ± 0.1	2.9 ± 0.1	2.0 ± 0.0	0.4 ± 0.02	1.3 ± 0.2	1.2 ± 0.2	1.4 ± 0.2	12.2 ± 0.9
O ₂	0.6 ± 0.1	0.8 ± 0.1	2.1 ± 0.3	0.9 ± 0.1	2.6 ± 0.3	8.4 ± 1.0	9.9 ± 1.2	11.2 ± 1.4	3.9 ± 0.2
H ₂	9.3 ± 1.8	5.1 ± 1.0	4.9 ± 0.9	5.3 ± 1.0	6.9 ± 1.3	11.6 ± 2.2	4.3 ± 0.8	4.1 ± 0.8	Not measured directly
<i>High-temperature H₂O (%)§</i>									
	21 ± 8	21 ± 8	26 ± 9	34 ± 11	26 ± 26	31 ± 8	19 ± 1	31 ± 11	
<i>Molar abundances (nmol)</i>									
HCl	58.5 ± 26.1	72.4 ± 32.2	536.1 ± 238.7	155.1 ± 69.1	44.1 ± 19.6	179.9 ± 80.1	267.7 ± 119.2	584.9 ± 260.5	36.5 ± 10.4
H ₂ S	79.0 ± 35.2	57.0 ± 25.4	95.2 ± 42.4	36.1 ± 16.1	18.7 ± 8.3	67.4 ± 30.0	38.9 ± 17.3	34.4 ± 15.3	61.0 ± 13.9
NO	190 ± 38	188 ± 38	162 ± 32	129 ± 26	190 ± 38	331 ± 66	389 ± 78	374 ± 75	175 ± 40
<i>Sample weight (%)</i>									
H ₂ O	2.3 ± 1.6	2.2 ± 1.5	2.4 ± 1.5	1.8 ± 1.2	1.7 ± 1.3	2.2 ± 1.5	2.5 ± 1.6	1.8 ± 1.2	2.0 ± 1.3
CO ₂	0.7 ± 0.3	0.8 ± 0.4	0.6 ± 0.2	0.6 ± 0.3	0.2 ± 0.09	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.1
SO ₃ equiv.	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.12	0.4 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.09	0.2 ± 0.10	2.0 ± 0.2
Cl ₂ O ₇ equiv.	0.07 ± 0.03	0.09 ± 0.04	0.24 ± 0.06	0.10 ± 0.04	0.3 ± 0.1	1.0 ± 0.4	1.2 ± 0.5	1.3 ± 0.5	0.4 ± 0.1

*Three sample portions (~135 ± 31 mg) of John Klein drill material were delivered to SAM for the JK-3 experimental run. The numbers in this column have been normalized to one sample portion (45 mg). †Average of four SAM runs of the Rocknest aeolian material (10). ‡Errors reported for molar abundances of CO₂, SO₂, and O₂ are the 2σ standard deviation from the mean of calculations done with different *m/z* values for the same species. H₂O error bars are based on the uncertainty in prelaunch water abundance calibration. Errors for other species include the uncertainty in differences in ionization efficiency between masses with a calibrated mol/counts value and uncalibrated values (10). Weight % values were calculated with an estimated sample mass of 45 ± 18 mg (2σ), with errors propagated including the uncertainty in molar abundance (10).

§High-temperature H₂O is the percentage of the H₂O released between 450° and 835°C, which is the temperature region for dehydroxylation of a 2:1 phyllosilicate, compared to the total H₂O released. Errors are the 2σ standard deviation from the mean.

and 2). The origin of the high-temperature evolved H_2 is unknown but is likely associated with the dehydroxylation of the most thermally stable OH groups in the 2:1 phyllosilicates.

Evolved O_2

The JK and CB samples have distinctly different O_2 releases (Figs. 1A and 2A and Table 1). The onset of O_2 evolution from JK ($\sim 150^\circ\text{C}$) was lower than for CB ($\sim 230^\circ\text{C}$). O_2 abundances released from CB (0.3 to 1.3 wt % Cl_2O_7) were nearly eight times the abundances for JK (0.07 to 0.24 wt % Cl_2O_7); Rocknest O_2 abundances (0.4 wt. % Cl_2O_7) were about four times those for JK (Table 1). By comparison, the perchlorate anion (ClO_4^-) was present in soil at the Phoenix landing site at the 0.4 to 0.6 wt % level (20). The JK-4 sample had two distinct peaks, suggesting different or additional O_2 -evolving phases in the JK sample or consumption of O_2 during combustion of organic materials (see below) or thermal oxidation of ferrous-containing phases (e.g., magnetite to maghemite transition). O_2 evolution in the Rocknest aeolian material occurred at a higher temperature (onset $\sim 300^\circ\text{C}$ with a peak temperature $\sim 400^\circ\text{C}$) than in JK and CB (10, 21).

Evolved O_2 from JK and CB is inferred to result from decomposition of perchlorate or chlorate salts, based on analogy with other analyses on Mars, on bulk compositions of the JK and CB

samples, on the timing of chlorinated hydrocarbon and HCl releases, and on laboratory experiments with perchlorate salts. Perchlorate was definitively identified in soil at the Mars Phoenix landing site (20), and O_2 release from the Rocknest aeolian material is roughly consistent with decomposition of Ca-perchlorate (10, 21). The CB sample contains three times the Cl of the JK sample as measured by APXS (1.41 wt % versus 0.4 wt % for CB and JK, respectively) (7), and the abundance of O_2 released from CB (splits CB-2 and CB-3) is nearly eight times the abundance from JK (Table 1), suggesting that the substance responsible for the release of O_2 from CB was a perchlorate or chlorate. Similarly, HCl and chlorinated hydrocarbons (chloromethane and dichloromethane) were released in conjunction with O_2 (Figs. 1 and 2, see below), suggesting that Cl and O were hosted by the same compound in CB and JK.

The coincident release of HCl with O_2 in CB is consistent with several types of perchlorate salt (Fig. 2). HCl is evolved during thermal decomposition of Mg- and Fe-perchlorate, caused by reaction between Cl_2 gas and water vapor (22–24). Thermal decomposition of Ca-perchlorate alone does not yield substantial HCl at temperatures $< 450^\circ\text{C}$ (22, 25). However, thermal decomposition of Ca-perchlorate in the presence of an Fe-bearing mineral such as pyrrhotite can also yield simultaneous releases of O_2 and HCl (Fig. 3B).

The O_2 -release profiles and temperatures for JK and CB do not match exactly those of common perchlorate salts. Although the best matches are with Fe-perchlorates (Fig. 3C), the presence of Fe-oxides/oxyhydroxides may lower the decomposition temperature of perchlorate salts (26). Chlorate salts may also be stable on the martian surface (27), and mixtures of K-chlorate and hematite can decompose at temperatures consistent with O_2 -release temperatures observed in JK and CB (26). Other possible sources of the low-temperature O_2 release—e.g., peroxides and superoxide radicals—cannot be ruled out (28–30).

Evolved CO_2

The CO_2 releases for the JK and CB samples peaked at temperatures below 300°C (Figs. 1A and 2A), distinct from the CO_2 release between 400° and 512°C from the Rocknest aeolian materials (10). The CO_2 releases in the Rocknest samples were interpreted to derive largely from carbonates (10), and the CO_2 release shoulder around 400° to 450°C in the JK samples could also derive from carbonate minerals, specifically fine-grained Fe/Mg-carbonate (10, 24, 31). The 400° to 450°C release shoulder is absent from the CB samples. Another possible CO_2 source, given the inferred presence of akaganeite and substantial proportions of perchlorate or chlorate phases in the samples, is that HCl evolved at lower temperatures and then reacted with carbonate minerals (23). The onset of evolved HCl is nearly simultaneous with CO_2 releases in JK and CB (Figs. 1 and 2), suggesting that low-temperature acid dissolution and subsequent thermal decomposition of carbonates may be responsible for some of the evolved CO_2 (fig. S2). Total CO_2 evolved is equivalent to < 1 wt % carbonate and, if present, carbonates are at abundance below the detection limit by CheMin. Adsorbed CO_2 is an unlikely candidate for the CO_2 peak near 300°C because most adsorbed CO_2 is expected to be desorbed from smectite and palagonite-like material surfaces at temperatures $< 200^\circ\text{C}$ (32). The low-temperature shoulder around 100° to 200°C in JK materials could reflect adsorbed CO_2 , although it was not seen in CB materials.

Although there are several possible CO_2 sources in JK and CB materials, the simultaneous evolution of CO_2 and O_2 , in conjunction with a possible O_2 inversion (i.e., O_2 consumption) in JK-4 and the similar CO_2 and O_2 releases in CB samples, suggest combustion of C compounds. It is nearly certain that at least some of the CO_2 produced is derived from the combustion of vapor from *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA, a derivatization agent carried in SAM) and its reaction products that were identified by the EGA and GCMS experiments and adsorbed onto the samples and sample cups inside SAM during sample transfer in Curiosity's sample acquisition and processing system (10, 21, 33). The background-derived C detected in the blank runs was up to ~ 120 and 30 nmol of C attributed to MTBSTFA and

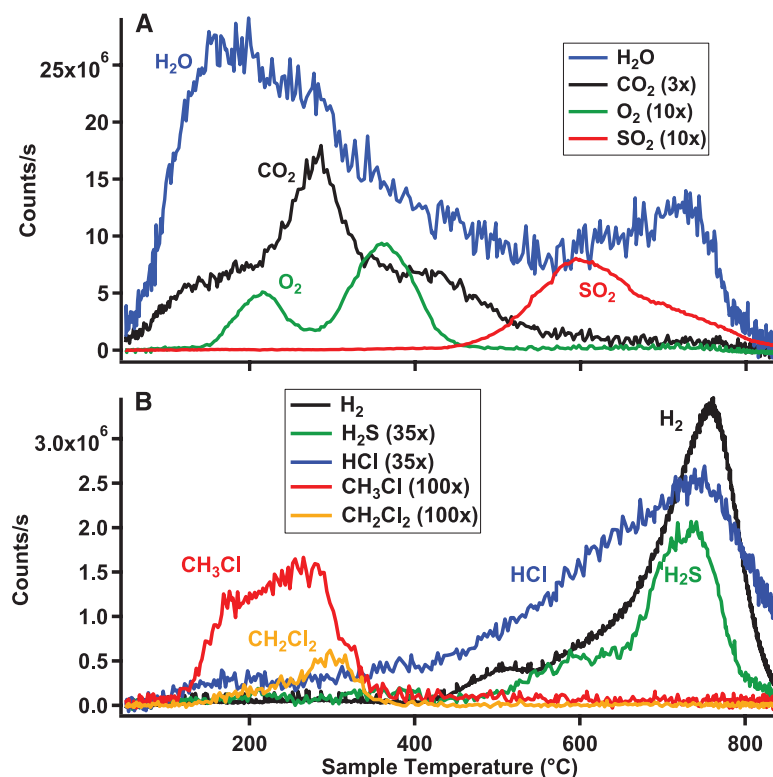


Fig. 1. Evolved gas analysis for a continuous ramp SAM pyrolysis of drill material (<150 μm) from the Sheepbed mudstone JK-4 sample. (A) Most-abundant evolved gases [CO_2 has been scaled up 3 times; SO_2 and O_2 have been scaled up 10 times]. (B) Evolved gas traces for H_2 , H_2S , HCl, CH_3Cl , and CH_2Cl_2 [H_2S and HCl traces have been scaled up 35 times; CH_3Cl and CH_2Cl_2 have been scaled up 100 times]. Isotopologs were used to estimate species that saturated the QMS detector ($m/z = 12$ for CO_2 and $m/z = 20$ for H_2O).

dimethylformamide (DMF) (33), respectively. If the background was similar for the analyzed samples of the mudstone, another source of C for combustion to CO₂ during pyrolysis is required to account for the >2 μmol of evolved CO₂ (Table 2). The estimated amount of MTBSTFA + DMF C in the blank runs is only 1 to 3% of total evolved CO₂-C from JK and CB analyses. Also, lower amounts of MTBSTFA C (~18 nmol C) and DMF C (~15 nmol C) were detected in the CB-5 analysis (Table 2), suggesting that substantially less MTBSTFA and DMF were available for combustion to CO₂ in this run due to implementation of the MTBSTFA-reduction protocol (14). These results indicate that most of the evolved CO₂ from CB is not related to the known terrestrial C background in SAM, and therefore additional C sources are required.

The initial amount of MTBSTFA and DMF C in the JK and CB analyses could have been higher than the levels measured in the empty-cup blank because of additional adsorption of these volatiles to the solid-sample surface area after sample delivery. A triple-sized sample portion (~135 mg of sample) of JK (JK-3) was delivered to SAM to explore the effects of adsorption on measured CO₂ releases. Estimates of the amount of C from MTBSTFA and DMF sources that could contribute to evolved CO₂ for the JK-3 triple-portion sample show that the levels are similar (within error) compared to the single-portion JK sample analyses (column 4 of Table 2), despite the potential for at least three times as much MTBSTFA and DMF adsorption to the sample due to greater surface area. Also, the triple-portion sample evolved 2.4 to 3.5 times as much CO₂ (Table 2). MTBSTFA and DMF C likely contributed to a small portion of the evolved CO₂ (21). The order-of-magnitude more C observed as CO₂ in all samples and the near threefold increase in CO₂ observed for the triple portion run further demonstrate that the dominant C source for CO₂ in the JK analyses came from the mudstone itself and not from known background C sources in SAM or Curiosity's sample acquisition and delivery system.

Another possible C source for the evolved CO₂ is combusted martian indigenous and/or exogenous (meteoritic) organic matter in the mudstone. The Sheepbed mudstone has trace-element compositions consistent with a meteoritic contribution that may have delivered 300 to 1200 parts per million (ppm) organic C (7) and/or from weathering of igneous material (34). Also, metastable partially oxidized weathering products of martian organics of indigenous and/or exogenous origins such as mellitic acid (35) may have undergone decarboxylation during analysis in SAM. Laboratory analog experiments using SAM-like instrument conditions where mellitic acid and perchlorate salts were heated together have shown that the primary degradation products detected during pyrolysis are CO₂ and CO (36, 37). If mellitic acid or other benzenecarboxylates were present in Sheepbed, the organic degradation products may have decomposed to CO₂ and other less-

volatile degradation products, which could have gone undetected by SAM GCMS under the oven conditions used for the pyrolysis experiments.

Overall, the potential CO₂ sources include combustion of terrestrial organics resident in SAM (for a small portion of the evolved CO₂), low-temperature acid dissolution of martian carbonates, and combustion and/or decarboxylation of indigenous and/or exogenous organic materials.

Evolved SO₂ and H₂S

Sulfur dioxide and H₂S evolved from the JK and CB samples during pyrolysis, and both also evolved from the Rocknest aeolian material (10). The release of both reduced and oxidized S volatiles suggests that reduced and oxidized S species were present in all samples, or that redox reactions in the SAM oven affected S speciation. The total abundance of S-bearing gases and the ratio of SO₂/H₂S observed at JK were both <30% of the values measured in Rocknest samples. Abundances of these gases were even lower in CB than in JK, consistent with the lower bulk S composition measured by APXS [1.57 (±0.03) wt % SO₃ for CB compared with 5.52 (±0.21) wt % SO₃ for JK (7)].

The evolved SO₂ had a release peak temperature at ~600° to 625°C, with a shoulder at ~675°C (Figs. 1A and 2A). Several mineral sources

of S are possible. CheMin detected anhydrite, bassanite, and pyrrhotite in both JK and CB and possible pyrite in JK (8). Pyrrhotite and possibly pyrite are candidate S sources for the SO₂ and H₂S releases from CB and JK. The lower-temperature SO₂ and HCl evolutions occurred simultaneously with the release of O₂ in CB (Fig. 2), and two additional SO₂ release peaks were observed in the 500° to 800°C range. Laboratory pyrolysis experiments of mixtures of pyrrhotite and Ca-perchlorate exhibited similar release patterns for SO₂, O₂, and HCl (Fig. 3B); however, the onset temperatures for their release is lower in CB, consistent with a different perchlorate/chlorate salt (i.e., lower O₂ release) or complex chemistry occurring in the SAM ovens that lowers the decomposition temperature of oxychlorine compounds (as discussed above). Thermal decomposition of Ca-sulfate is not likely to have contributed to the SO₂ releases from JK and CB because they typically break down at higher temperatures than the maximum that can be achieved by the SAM oven used in these experiments (>835°C).

Although pyrrhotite and pyrite are candidate S-bearing phases in CB and JK, the evolved SO₂ data are not uniquely diagnostic of these or any specific S-bearing phases. Fe-sulfate minerals

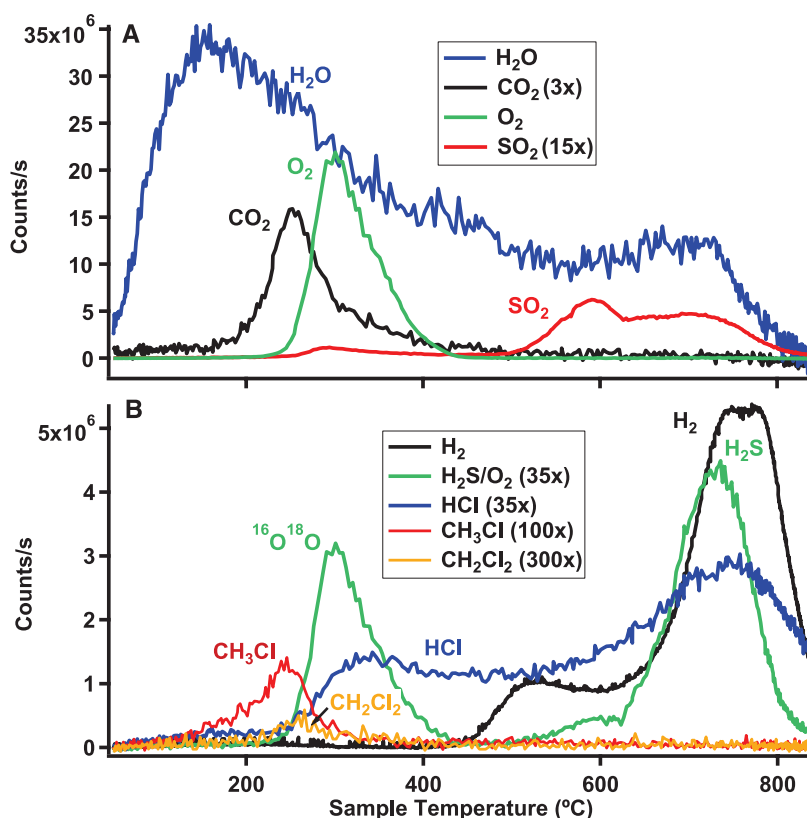


Fig. 2. Evolved gas analysis for a continuous ramp SAM pyrolysis of drill material (<150 μm) from the Sheepbed mudstone CB-2 sample. (A) Most-abundant evolved gases [CO₂ and SO₂ counts have been scaled up 3 and 15 times, respectively]. (B) Evolved gas traces for H₂, H₂S, HCl, CH₃Cl, and CH₂Cl₂ [H₂S and HCl traces have been scaled up 35 times; CH₃Cl and CH₂Cl₂ have been scaled up 100 and 300 times, respectively]. Isotopologs were used to estimate species that saturated the QMS detector ($m/z = 12$ for CO₂ and $m/z = 20$ for H₂O).

will evolve SO_2 at 500° to 800°C under conditions similar to SAM operational conditions. However, formation of Fe-sulfates requires strongly acidic conditions (38, 39), and Sheepbed is interpreted to record depositional and diagenetic environments at near-neutral pH's (1).

Evolution of H_2S occurred nearly simultaneously with evolution of H_2 and high-temperature H_2O resulting from the dehydroxylation of the 2:1 phyllosilicate (Figs. 1 and 2). H_2S may be a by-product of the reaction of H_2O with a Fe-sulfide such as pyrrhotite (Fig. 3B); however, it is possible that SO_2 evolved at high temperatures is reduced in the presence of H_2 to H_2S (40, 41). HCl also evolves at higher temperatures (Figs. 1B and 2B), which can react with reduced S phases to form H_2S (42, 43).

Evolved N-Bearing Species

Potential N-bearing compounds evolved from JK and CB include NO, HCN, CH_3CN , ClCN , CF_3CN , and $\text{C}_3\text{H}_4\text{F}_3\text{NO}$. Evolved NO [mass/charge ratio (m/z) = 30] in the JK and CB materials had abundances of 129 to 190 nmol and 190 to 389 nmol NO, respectively (Table 1). The abun-

dances of NO in JK and CB blank runs were 70 and 12 nmol, respectively, and the predicted level of N contributed by MTBSTFA and DMF based on background measurements was typically less than 20 nmol N. Hence, the source for the evolved NO appears to be within the mudstone. Other N compounds detected by SAM (HCN, CH_3CN , and ClCN) are present at substantially lower abundances, and they may be contributed by the MTBSTFA and DMF background in the SAM instrument. Both wet chemistry reagents contain one N atom per molecule of reagent. CF_3CN is almost certain to be from decomposition of MTBSTFA because possible sources of F in the martian samples (i.e., fluorapatite) will not decompose in the SAM temperature range. In addition, HCN and CH_3CN have also been identified during laboratory pyrolysis of MTBSTFA and DMF in the presence of perchlorate run under similar operating conditions as SAM (21).

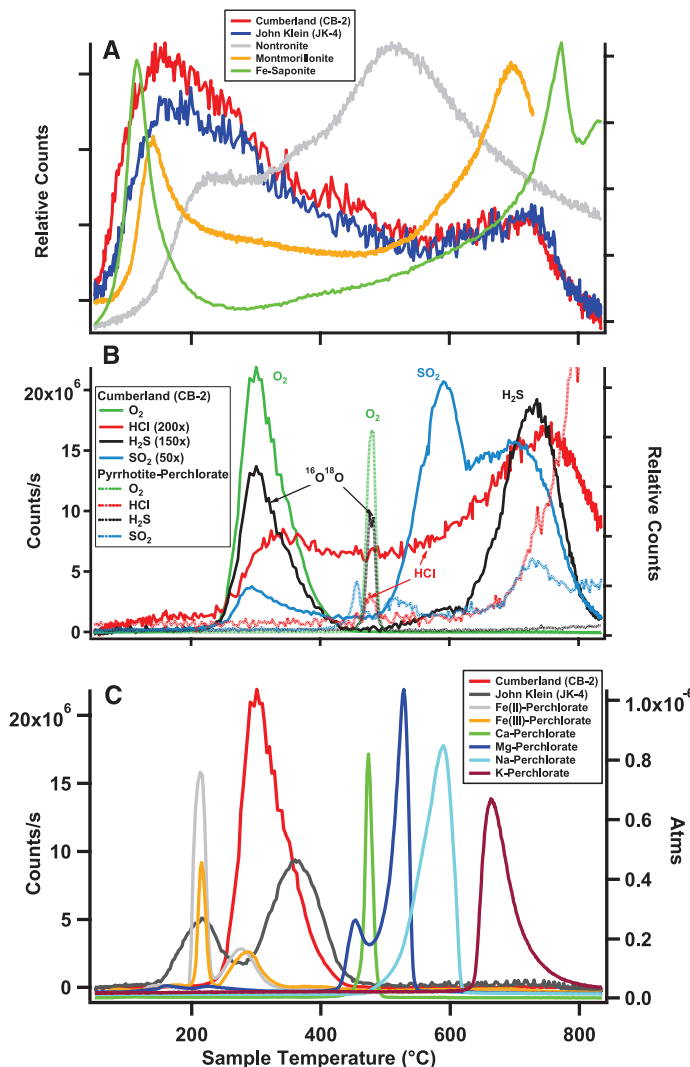
Organic Compounds

Pyrolysis of the JK and CB samples led to low-temperature (125° to 350°C) release of chloromethane and dichloromethane that correlated with

the release of O_2 and 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyldisiloxane, a known reaction product of MTBSTFA and H_2O (fig. S3). This correlation suggests that thermal degradation of the O_2 source (most likely an oxychlorine compound, see above) is contributing to chlorination of C phases and/or the release of chlorinated hydrocarbons. Identification and quantification of these trace organic species by their characteristic m/z values in EGA mode was enhanced and confirmed by bulk collection and GCMS identification (44) of volatiles on an adsorbent-resin hydrocarbon trap (Fig. 4 and fig. S4). Detection of chlorinated hydrocarbons by SAM in Rocknest samples (10, 21), as well as supporting laboratory EGA and GCMS experiments conducted under SAM-like conditions, have shown that both chloromethane and dichloromethane are produced when MTBSTFA and DMF are heated in the presence of Ca- and Mg-perchlorates (21). Therefore, reaction of MTBSTFA and DMF carbon with an oxychlorine compound is a likely source of some of the chloromethane and dichloromethane detected in JK and CB.

Empty-cup blank analyses before each sample set (Rocknest, JK, and CB) showed low chloromethane C abundance (<7 nmol C, including estimated error, via EGA) and no detection of dichloromethane. In comparison to the blanks, single-portion runs of JK and CB consistently showed more abundant chloromethane and dichloromethane C [up to about six times the abundance; range: $21 (\pm 4)$ to $66 (\pm 13)$ nmol C, Table 2], whereas lower amounts of chloromethane and dichloromethane [$12 (\pm 2)$ nmol C] were released from Rocknest single-portion samples, even though the total MTBSTFA C amounts measured during pyrolysis of Rocknest were similar (within error) to those measured in JK and the CB-1 and CB-2 runs (Table 2). Furthermore, the JK-3 EGA analysis of a triple-portion sample released approximately twice as much chloromethane + dichloromethane [$127 (\pm 25)$ nmol C] as a single-portion JK analysis [$38 (\pm 8)$ to $66 (\pm 13)$ nmol C], and JK-3 released trichloromethane (CHCl_3) and carbon tetrachloride (CCl_4), which were not previously detected in the blank or single-portion JK experimental runs (table S2 and Fig. 4). However, the increase in chlorinated products in JK-3 could also be explained by three times as much sample (i.e., more oxychlorine compounds) compared to the single-portion JK runs. Trichloromethane and carbon tetrachloride were also detected by GCMS in both CB-3 and CB-5 at similar levels (table S2). These data indicate that greater chlorinated hydrocarbon production is associated with the Sheepbed mudstone (compared with the Rocknest aeolian deposit) and with larger sample mass. However, the substantial reduction ($\sim 50\%$) in C abundance from MTBSTFA (and presumably DMF) in the CB-5 experiment compared with CB-3 (fig. S5 and Table 2) was matched by a $\sim 50\%$ drop in chloromethane and dichloromethane detected by EGA [$10.2 (\pm 2.0)$ and $0.7 (\pm 0.2)$ nmol C, respectively]. This suggests that

Fig. 3. Major evolved gases from the JK and CB samples compared with EGA of analog minerals under SAM-like oven operating conditions (14). (A) Evolved H_2O for nontronite, montmorillonite, and saponite compared with JK and CB evolved H_2O . (B) Evolved O_2 , SO_2 , HCl , and H_2S for CB-2 compared with pyrrhotite mixed with a Ca-perchlorate run under SAM-like operating conditions in laboratory experiments (HCl evolution coincides with O_2 release for CB-2 and laboratory pyrrhotite/perchlorate experiments). (C) Evolved O_2 for several perchlorate salts compared with JK and CB evolved O_2 .



martian organics may not be substantial contributors to these chloromethanes detected in CB samples.

Trace quantities of chloromethane and dichloromethane were also identified by the Viking 1 and 2 lander GCMS instruments (45, 46). The Viking GCMS team attributed the chlorinated hydrocarbons to terrestrial sources including cleaning solvents, although the possibility that some of the chloromethane C was indigenous to Mars was not ruled out (46). Unlike SAM, the Viking GCMS instruments did not include an MTBSTFA and DMF wet chemistry experiment. Following the Phoenix perchlorate detection and subsequent laboratory experiments in which Atacama soils mixed with 1 wt % of Mg-perchlorate produced chloromethane and dichloromethane at 500°C, the Viking chloromethane compounds have been attributed to the presence of perchlorates and indigenous organic carbon at the Viking landing sites (47). The hypothesis has been challenged (48) and debated (49).

The EGA and GCMS observations of varying chlorinated hydrocarbon abundances in JK and CB could result from any combination of the following: (i) chlorination of MTBSTFA and DMF or other unknown terrestrial C sources in SAM (instrument background) that were not identified during EGA or GCMS in the empty-cup blank runs; (ii) chlorination of C contamination from the drill and/or sample handling chain; and (iii) chlorination of martian or exogenous C phases in the Sheepbed mudstone.

Terrestrial contamination from the sample handling chain is unlikely because it was scrubbed multiple times with Rocknest scooped material before the first drilled sample at JK (9, 10). The

GCMS abundances of chloromethane and dichloromethane measured in JK-4 were similar within error to the abundances of the chlorinated hydrocarbons identified in the second drill sample at CB (CB-2) run under identical conditions (table S2). Therefore, if terrestrial C contamination from the drill was the primary source of C for these chlorinated hydrocarbons in JK-4, their abundances measured in the CB-2 sample should have been reduced compared to JK-4 due to sample scrubbing of hardware surfaces and disposal of the JK sample from the sample processing system. This was not observed. Moreover, swabbed surfaces of Curiosity's sample acquisition and processing system were found to be organically clean before launch (50, 51), and only trace quantities (~0.1 to 0.3 nmol) of perfluoroethene, a known pyrolysis product of Teflon that is within the drill system (52), were identified in the JK and CB EGA analyses.

Chlorinated hydrocarbons are likely produced in the SAM oven during heating of samples in the presence of Cl from the thermal decomposition of oxychlorine compounds. Some of the chlorinated hydrocarbon detected at JK and CB may be derived from the sample. The SAM data do not allow us to prove, or disprove, organic C contributions from Sheepbed to evolved chlorinated hydrocarbons and CO₂ from the JK and CB samples. There are no conclusive EGA or GCMS observations of other organic molecules indigenous to the Sheepbed mudstone.

Preservation of Organics

Although the detection and identification of possible organic compounds in the Sheepbed mudstone is complicated by reactions in the SAM oven,

the lack of a definitive detection of martian organics suggests that, if organics were deposited in the Sheepbed mudstone, organic alteration and destruction mechanisms may present the single most fundamental challenge to the search for organics on Mars. Some organic compounds are expected on the surface of Mars. Exogenous delivery of meteoritic organics to the martian surface has been estimated as 2.4 × 10⁸ g C/year (53) and may have been higher during periods of higher impact flux on the surface. Benzenecarboxylates derived from the oxidation of meteoritic organic matter on Mars could contribute up to 500 ppm organic C by weight in the top meter of the martian regolith (35). Abiotic organic matter formed by igneous and/or hydrothermal processes (34, 54) is also expected to be embedded within basaltic minerals where it is protected from chemical oxidants in the environment. Consequently, at least a low concentration of organic compounds is likely in the source material for the Sheepbed mudstone. In addition, the distal fluvial to lacustrine depositional environment of Sheepbed (1) makes it a prime site for concentration of organic matter through sedimentary processes (2).

If organics were present, evaluating their fate during diagenesis becomes critical for understanding their molecular structures, distribution in sediments, and SAM observations. The JK and CB samples experienced diagenesis, including the alteration of basaltic minerals to smectite and magnetite after deposition (7, 8). Organics may have been released during basaltic mineral alteration, and any reactive organic molecules present may have been incorporated into or adsorbed onto the forming smectite and magnetite. These types of mineral-organic associations could have enhanced

Table 2. Abundances of terrestrial C from *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) reaction products and dimethylformamide (DMF) compared with the measured abundances of chloromethane (CM), dichloromethane (DCM) and CO₂ detected during SAM evolved gas analysis (EGA) by the mass spectrometer.

Sample	Total detected MTBSTFA-derived C (μmol)*	Estimate of missing DMF C (μmol)†	Total CM + DCM C (μmol)	Estimated amount of MTBSTFA- and DMF-derived C for combustion (μmol)‡	Total CO ₂ (μmol)	Estimated MTBSTFA + DMF C combustion in analysis relative to total CO ₂ §
JK blank	0.120 ± 0.024	0.030 ± 0.006	0.005 ± 0.001	≈ 0	≈ 0	≈ 0%
JK-1	0.051 ± 0.010	0.013 ± 0.003	0.066 ± 0.013	0.086 ± 0.043	7.0 ± 1.9	1.2 ± 0.7%
JK-2	0.044 ± 0.009	0.011 ± 0.002	0.061 ± 0.012	0.095 ± 0.041	8.1 ± 1.7	1.2 ± 0.5%
JK-3 (3× portion)	0.071 ± 0.014	0.018 ± 0.004	0.127 ± 0.025	0.061 ± 0.048	19.8 ± 4.8	0.3 ± 0.3%
JK-4	0.047 ± 0.009	0.012 ± 0.002	0.038 ± 0.008	0.091 ± 0.041	5.7 ± 1.3	1.6 ± 0.7%
CB blank	0.097 ± 0.019	0.025 ± 0.005	0.006 ± 0.001	≈ 0	≈ 0	≈ 0%
CB-1	0.068 ± 0.014	0.017 ± 0.003	0.025 ± 0.005	0.037 ± 0.041	2.0 ± 0.2	1.9 ± 2.1%
CB-2	0.041 ± 0.008	0.010 ± 0.002	0.022 ± 0.004	0.071 ± 0.034	2.5 ± 0.4	2.8 ± 1.4%
CB-3	0.032 ± 0.006	0.008 ± 0.002	0.021 ± 0.004	0.082 ± 0.032	3.1 ± 0.3	2.7 ± 1.1%
CB-5	0.018 ± 0.004	0.005 ± 0.001	0.011 ± 0.002	ND	3.1 ± 0.7	ND
RN blank	0.078 ± 0.016	0.020 ± 0.004	0.002 ± 0.001	≈ 0	≈ 0	≈ 0%
RN1-4 (average)	0.058 ± 0.012	0.015 ± 0.003	0.012 ± 0.002	0.025 ± 0.035	9.9 ± 1.2	0.3 ± 0.4%

*Total MTBSTFA C value in μmol determined from the sum of the EGA measured abundances of silylated products: *tert*-butyldimethylsilyl, 1,3-bis(1,1-dimethylethyl)-1,1,3,3-tetramethyldisiloxane, and *tert*-butyldimethylfluorosilane, plus the mole fraction of C from 2,2,2-trifluoro-*N*-methylacetamide in MTBSTFA relative to the sum of the silylated products. †DMF was not identified during pyrolysis by EGA or GCMS. However, the amount of DMF C was estimated from the total measured MTBSTFA C and the molar ratio of DMF/MTBSTFA carbon (0.25) that was originally loaded into the wet chemistry cups, to address a worst-case scenario. ‡Estimated amount of combusted MTBSTFA- and DMF-derived C equals the amount of C in the blank from these sources minus the amount of C from these sources that was observed and calculated for each solid sample run. §Percentage of the total MTBSTFA and DMF carbon in the preceding blank analysis, assumed to be representative of the MTBSTFA and DMF derived carbon available for combustion to CO₂, compared to total CO₂ measured in the sample runs. ||ND: Values could not be determined because a comparable blank run was not carried out under the same experimental conditions that were used for CB-5.

the early preservation of organic matter. In addition, reducing conditions during deposition, as suggested by the presence of magnetite (7), would have favored early preservation. Subsequently, and at any time during burial, oxidants already present in disequilibrium with reduced phases or circulated into the rock may have contributed to the oxidative degradation of organic compounds that were deposited with the sediment or released from the altering igneous minerals. There is no evidence of mineral oxidation associated with the second diagenetic event that precipitated calcium sulfate minerals from fluids into the Sheepbed fracture network (7), and the bulk rock remained largely reducing based on the presence of magnetite and pyrrhotite in JK and CB, and possibly pyrite in JK (8). However, akaganeite in the samples may reflect oxidative

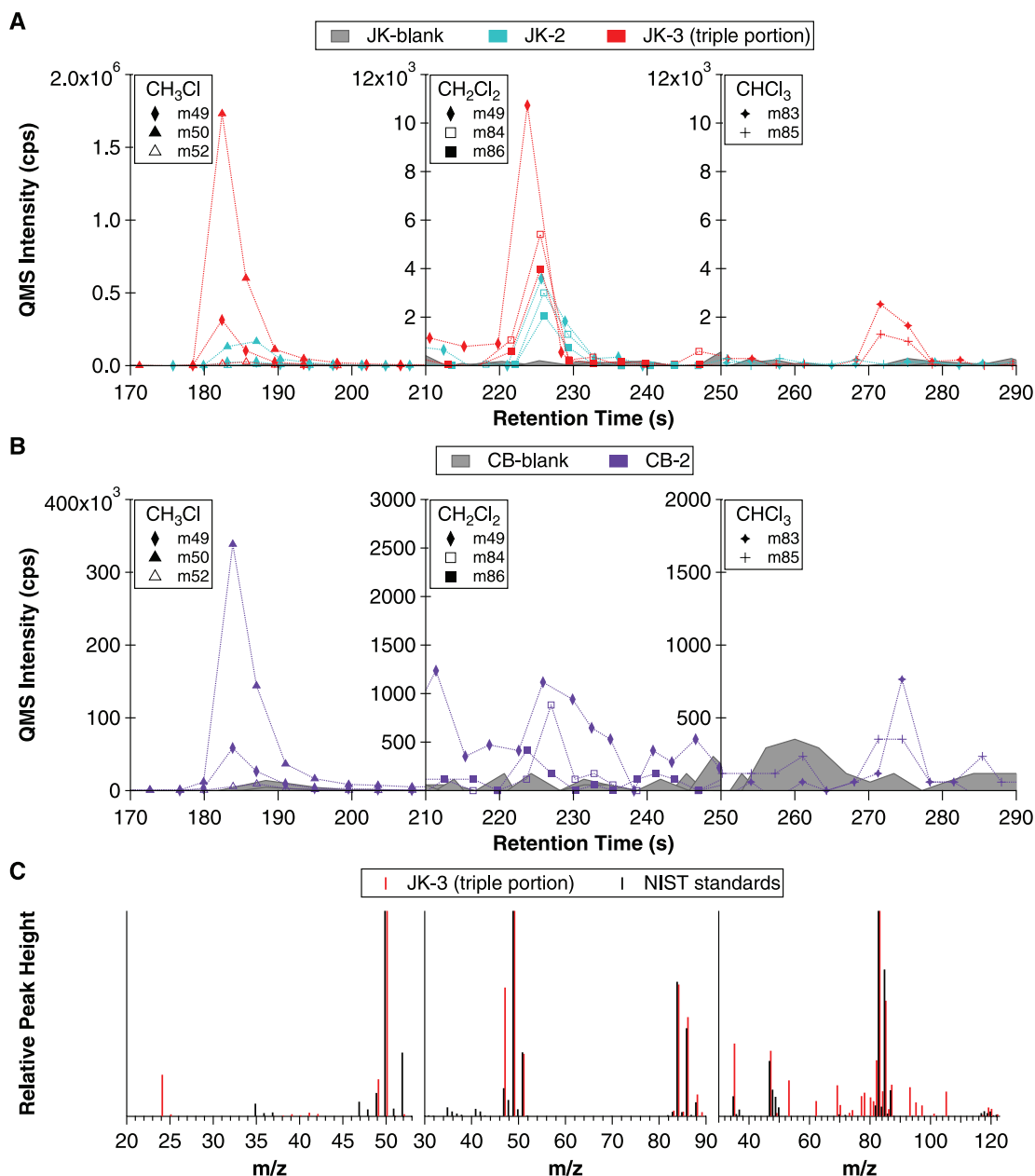
weathering of pyrrhotite, which could indicate exposure of JK and CB to an oxidizing fluid in earlier diagenetic event(s) that may have degraded any organic compounds.

If any organic compounds remained throughout burial, they may have been altered by other mechanisms. The martian surface is subjected to strong ionizing radiation that can alter organic molecules (55) depending on the chemical and physical microenvironment of the host sediments (56). Ionizing radiation directly breaks chemical bonds in organic molecules and other chemicals, producing a reactive pool of radicals and oxidants (e.g., $\text{OH}\cdot$, H_2O_2 , oxychlorine compounds). In the presence of mineral catalysts, these reactants can fully oxidize organic matter to CO , CO_2 , and carbonates or produce partially oxidized organics such as acetate, oxalates, and other carboxylates

that may survive in a metastable state on Mars (35). If exogenous or martian organic matter survived largely intact until removal of the overlying Gillespie sandstone, it may still have degraded or oxidized during surface exposure of the sampling site to ionizing radiation. Therefore, surface-exposure age is also an important variable in the preservation of organics. Alternatively, the organics may have survived all of the martian processing that occurred naturally, only to be oxidized by oxide minerals or oxychlorine compounds at elevated temperatures in the SAM oven or, if sufficiently refractory, survive pyrolysis and pass undetected by SAM, as does most of the kerogen-like material in meteorites.

The complicated story of carbon on Mars is poorly understood. As of sol 370, SAM results support the presence of carbon source(s) in

Fig. 4. SAM gas chromatogram of the major m/z values of the chlorinated hydrocarbons detected in JK and CB samples (cps, counts/s). (A) JK Sample 3 (JK-3; triple portion) compared with JK-2 (single portion) and the JK blank. (B) CB Sample 2 (CB-2, single portion) compared with the CB blank. (C) The mass spectra generated for the GC peaks detected in JK-3 are shown in red and compared with the mass spectra for chloromethane (CH_3Cl), dichloromethane (CH_2Cl_2), and trichloromethane (CHCl_3) in black from NIST/EPA/NIH Mass Spectral Database (44).



samples from the Sheepbed mudstone that contribute(s) to the production of chlorinated hydrocarbons and evolved CO₂. There may be organic matter in these samples, but it has not been confirmed as martian.

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Acknowledgments: We are indebted to the Mars Science Laboratory Project engineering and management teams for making this mission possible and enhancing science operations. Much of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under contract with the National Aeronautics and Space Administration (NASA). NASA provided support for the development of SAM. Data from these SAM experiments are archived in the Planetary Data System (pds.nasa.gov). Essential contributions to the successful operation of SAM on Mars and the acquisition of this data were provided by the SAM development, operations, and testbed teams. Development and

operation of the SAM and APXS instruments were also supported by funds from the French Space Agency (CNES) and the Canadian Space Agency. Work in the UK was funded by the UK Space Agency. B.L.E., J.L.E., K.F., D.P.G., J.E.G., K.E.M., S.M.M., J.M., P.B.N., and R.E.S. acknowledge funding support from the NASA ROSES MSL Participating Scientist Program.

Supplementary Materials

www.sciencemag.org/content/343/6169/1245267/suppl/DC1

Materials and Methods

Figs. S1 to S6

Tables S1 to S3

References (57–65)

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28 August 2013; accepted 12 November 2013

Published online 9 December 2013;

[10.1126/science.1245267](https://doi.org/10.1126/science.1245267)

In Situ Radiometric and Exposure Age Dating of the Martian Surface

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We determined radiogenic and cosmogenic noble gases in a mudstone on the floor of Gale Crater. A K-Ar age of 4.21 ± 0.35 billion years represents a mixture of detrital and authigenic components and confirms the expected antiquity of rocks comprising the crater rim. Cosmic-ray-produced ³He, ²¹Ne, and ³⁶Ar yield concordant surface exposure ages of 78 ± 30 million years. Surface exposure occurred mainly in the present geomorphic setting rather than during primary erosion and transport. Our observations are consistent with mudstone deposition shortly after the Gale impact or possibly in a later event of rapid erosion and deposition. The mudstone remained buried until recent exposure by wind-driven scarp retreat. Sedimentary rocks exposed by this mechanism may thus offer the best potential for organic biomarker preservation against destruction by cosmic radiation.

Multiple orbiter and rover missions have documented a rich geologic record on the surface of Mars, likely spanning almost the entire history of the planet [e.g., (1)]. However, interpretation of this record is impeded by our limited understanding of the connection between the materials observed and their absolute age. Impact crater densities are the primary means for establishing a chronology for geologic features on Mars and other

solar system bodies (2–4), but crater-based dating methods suffer from multiple sources of uncertainty that obscure both absolute and relative ages. In contrast, on Earth, isotopic dating methods provide a powerful quantitative framework for defining geologic history and for identifying the rates and causes of geologic phenomena. Here, we report results of an attempt to apply in situ isotopic dating methods to Mars using the instrument suite aboard the Curiosity rover exploring Gale Crater.

Geologic Setting

Gale is a ~150-km diameter impact crater that formed near the Late Noachian–Early Hesperian boundary (5, 6) or around 3.7 to 3.5 billion years ago (Ga) according to impact crater models (3, 7). In addition to the ~5-km-high central mountain of stratified rock informally known as Mt. Sharp, the crater is partially filled with sedimentary rocks derived from the crater rim. Crater-density distributions imply a somewhat younger age (Early to Late Hesperian or ~3.5 to 2.9 Ga) for at least some of these deposits (5, 6). While heading for its destination at Mt. Sharp, Curiosity traversed a gently sloping plain where local outcrops of pebble conglomerate were encountered, recording the presence of an ancient stream bed (8). Most of this surface is heavily cratered and covered with rock and soil (Fig. 1). However, a substantial expanse of bare bedrock was encountered in an ~5-m-deep topographic trough (Yellowknife Bay) representing an erosional window through a sequence of stratified rocks known as the Yellowknife Bay formation (fig. S1) (9). These rocks consist mostly of distal alluvial fan and lacustrine facies of basaltic bulk composition and were derived

from the crater rim (9, 10). Compared with adjacent geological units, the Yellowknife Bay trough is notable for its lack of visible craters and its apparently greater degree of stripping of impact ejecta and wind-blown soil. This appearance suggests active erosion, distinctly different from the adjacent map units (9). However, the depositional age of the Yellowknife Bay formation, its stratigraphic relationship to the adjacent alluvial fan and to the strata of Mt. Sharp, and the causes and timing of its exposure are all presently uncertain.

As shown in Fig. 1, the Sheepbed mudstone and stratigraphically overlying Gillespie Lake sandstone of the Yellowknife Bay formation (9) form a rock couplet of apparently variable rock hardness, and their contact has eroded to form a decimeter-scale topographic step. The Sheepbed–Gillespie Lake contact is sharp and marked by scouring of the underlying mudstone, producing a small scarp and in some locations an overhang. Calved-off blocks of the sandstone occur at the base and a few meters outboard of the scarp but not beyond [see figure 3 of (9)]. This suggests that residual fragments of the degrading scarp are removed efficiently enough to leave only a very thin lag deposit at locations where the Gillespie Lake member is now completely absent.

The distinctive erosional profile of the Sheepbed–Gillespie Lake contact can be traced around the full width of Yellowknife Bay (9), with the Sheepbed unit making up the floor of the encircled trough. This trough is elongated in the northeast-southwest direction, creating an amphitheater-shaped planimetric form open to the northeast. Beyond the Gillespie Lake contact, the more-resistant units higher in the section form similar scarps stepping upward a total of ~5 m to the uppermost unit of the Yellowknife Bay formation (fig. S1). Within the Sheepbed unit, mm- to cm-scale topographic protrusions form fields of small ridges that have been streamlined in the northeast-southwest direction (Fig. 2). Along with the morphology of the Yellowknife Bay trough, these features indicate a dominant role for southwest-directed wind erosion. These observations implicate eolian processes in eroding the mudstone, in efficiently wearing down any blocks that are delivered to its surface from the encircling scarps or from distal impacts, and in expanding the exposure of the Yellowknife Bay formation.

Scientific investigations at Yellowknife Bay focused on the Sheepbed mudstone. This fine-grained (<50 μm) rock was drilled twice for mineralogical and evolved gas analyses (11, 12). In addition, multiple chemical analyses were obtained on outcropping mudstone as well as the drill tailings. The two drilled samples, referred to as John Klein and Cumberland, were located ~3 m apart and yielded very similar results. The mudstone is composed of detrital and authigenic components consisting of both crystalline and x-ray amorphous phases (11).

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Exploring Martian Habitability

The detrital fraction includes minerals typical of volcanic rocks: plagioclase; pyroxenes; and minor olivine, sanidine, and ilmenite. Crystalline authigenic phases include smectitic clay (possibly saponite) and magnetite, thought to have formed as a result of chemical alteration of detrital olivine, and minor akaganeite, pyrrhotite, bassanite, and hematite. Quartz and halite were reported but very near the Chemistry and Mineralogy instrument (CheMin) detection limit. Chlorate or perchlorate was also tentatively identified (12). A model for the amorphous component, comprising about one-third of the sample, indicates that it is composed mainly of Si, Fe, Ca, S, and Cl (11). It likely includes poorly crystalline or finely crystalline materials and may include detrital volcanic and impact-derived glass. The presence of smectite, magnetite, and akaganeite suggests that the mudstone has not experienced burial heating above $\sim 200^{\circ}\text{C}$ (11, 13). It is possible that the sample experienced no burial heating at all.

Geochronology Overview: K/Ar and Cosmogenic Isotopes

Noble gas isotopes can quantitatively constrain the age and erosion history of the Sheepbed mudstone. Argon-40 from ^{40}K decay will record a potassium-weighted average of the formation or cooling ages of the multiple components of the mudstone. Isotopes ^{36}Ar , ^{21}Ne , and ^3He are

produced by irradiation of the uppermost ~ 2 to 3 m of the martian surface by galactic cosmic rays (GCRs) (14). These cosmogenic isotopes are commonly used to assess exposure histories of meteorites [e.g., (15, 16)] and erosion rates and styles of terrestrial rocks [e.g., (17)]. Because the martian atmosphere is very thin and the magnetic field very weak (18), the martian surface is only weakly shielded from GCRs. Thus cosmogenic isotope-production rates are much higher than on Earth (14, 19). The chemistry of the Sheepbed mudstone (10) is such that the most abundant cosmogenic isotope is likely to be ^{36}Ar produced from the capture of cosmogenic thermal neutrons by Cl; followed by ^3He produced by spallation mainly of O, Si, and Mg; followed by ^{21}Ne from spallation of Mg, Si, and Al (20). The mudstone's exposure history is thus recorded by multiple isotopic systems.

The depth dependence of the production functions of the two spallation isotopes are very similar: a small maximum at ~ 15 cm below the surface, reflecting development of the nuclear cascade in the uppermost layers of rock, followed by an exponential decay over 2 to 3 m as the energetic particles attenuate (Fig. 3). In contrast, the production rate of ^{36}Ar from neutron capture has a larger subsurface maximum at greater depth (~ 60 cm), arising from the combined

effects of the production of neutrons within the descending cascade and the loss of low-energy neutrons into the martian atmosphere [e.g., (19)].

The concentration of a single cosmogenic isotope yields a nonunique exposure history for the rock. The customary end-member interpretive models are to assume either no erosion, in which case a surface exposure age is obtained, or steady erosion from great depth, in which case a mean erosion rate is obtained [e.g., (17)]. Figure 3 shows that this nonuniqueness can be eliminated by combining spallogenic and neutron-capture isotope measurements. In particular, a rock of Sheepbed composition initially at a depth of greater than a few meters that was instantaneously exposed at the surface would have $^{36}\text{Ar}/^3\text{He}$ of ~ 1.5 and $^{36}\text{Ar}/^{21}\text{Ne}$ of ~ 13 . In contrast, if steady erosion causes progressive downward migration of the surface toward the sample, then the sample integrates the entire depth profile, including the large subsurface ^{36}Ar peak. Such a rock would have $^{36}\text{Ar}/^3\text{He} \sim 4$ and $^{36}\text{Ar}/^{21}\text{Ne} \sim 30$. In both erosion scenarios, the $^3\text{He}/^{21}\text{Ne}$ ratio is ~ 8 ; this isotope pair provides a cross-check but no additional information on exposure history.

Results and Discussion

Geochronology measurements were performed on the Cumberland drilled powder (21). After

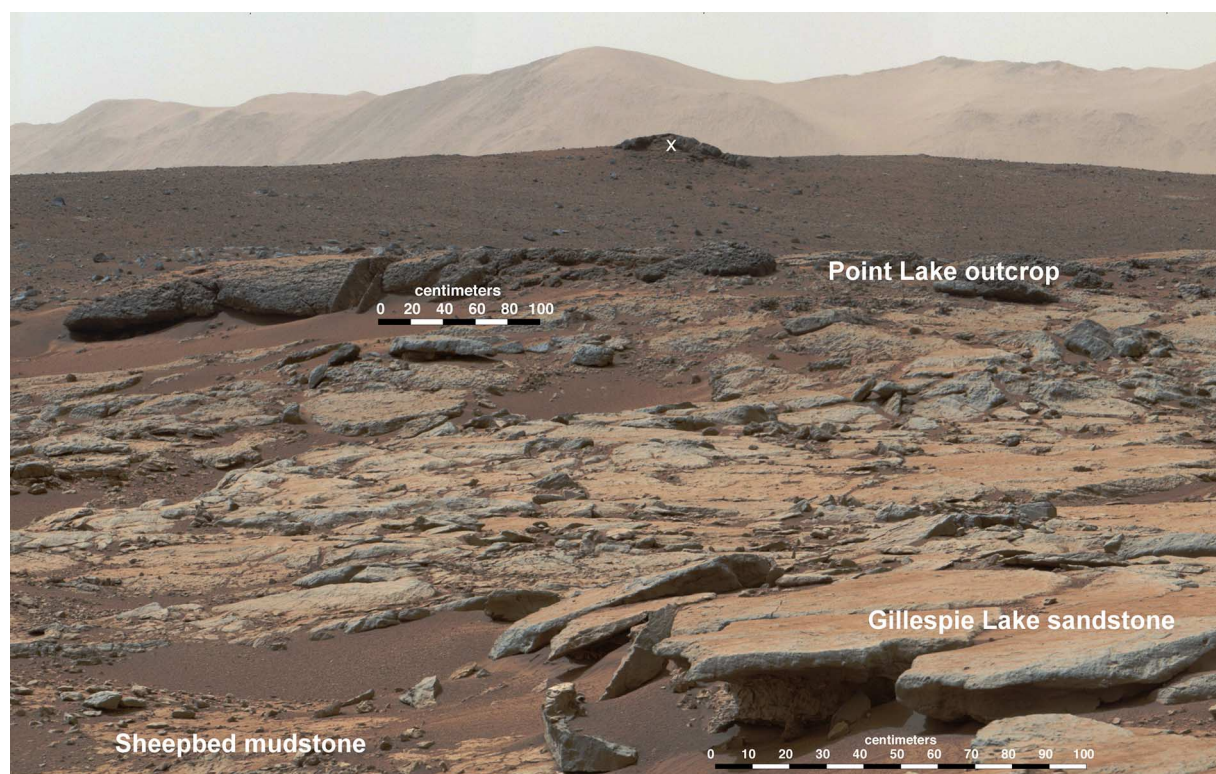


Fig. 1. Mastcam view looking 79° west of north. With increasing distance, the Yellowknife Bay formation includes the Sheepbed mudstone, Gillespie Lake sandstone, and Point Lake outcrop (36 m away). In the distance, the mostly rock- and sand-covered Bradbury rise is visible. The large outcrop on the near horizon (marked "x") is 240 m distant and stands 13 m above the Gillespie Lake–

Sheepbed contact. This is a portion of a 40-frame M-100 mosaic taken on sol 188 between 13:27 and 13:48 local mean solar time (14 February 2013 23:41:35 and 15 February 2013 00:03:23 Pacific Standard Time). The images in the full mosaic, acquired as sequence mcam01009, have picture identifications between 0188MR0010090000202415101 and 0188MR0010090390202454101.

drilling, the sample was sieved, and an aliquot with estimated mass of 135 ± 18 mg (22) was introduced into a quartz cup in the Sample Analysis at Mars (SAM) instrument. The cup was moved into position in an oven, sealed against Mars atmosphere, and evacuated. The sample was then heated to a maximum temperature of $\sim 890^\circ\text{C}$ for 25 min, and the evolved gases were

purified and admitted into the quadrupole mass spectrometer for analysis. All reported measurements used the high-sensitivity semistatic analysis mode.

K-Ar Age

The Ar concentration and the Alpha Particle X-ray Spectrometer (APXS)-determined K_2O con-

centration yield a K-Ar age of 4.21 ± 0.35 Ga (1σ) for the Cumberland sample (Table 1). This age calculation assumes that all measured ^{40}Ar is radiogenic (23). Although a fairly uniform level of excess Ar is thought to be present in some young martian meteorites (24), that same level of excess would contribute <0.03 billion years to the age of this high K_2O , high Ar concentration rock. One important implication of this very high K-Ar age is that processing in the SAM oven has extracted essentially all radiogenic ^{40}Ar despite a fairly low extraction temperature. This unexpectedly high yield (25) may result from rapid diffusive loss from the inherently fine grain size of the mudstone [probably enhanced by the powdering process of the drill (26)]. Alternatively, the volatile constituents in the mudstone may promote loss via flux-induced melting. Because fine grain size and flux melting will affect the noble gas isotopes similarly, effective extraction of ^{36}Ar , ^{21}Ne , and ^3He might also be expected. The high age also implies that a very large fraction of the radiogenic Ar was retained in the mudstone over geologic time.

The interpretation of the K-Ar age depends critically on where potassium is located in the rock. If a fraction F_D of the potassium is in the detrital phases and the remainder in authigenic phases, then the bulk age (T_B) is a potassium-weighted average of the age of the detritus (T_D) and the age of authigenesis (T_A): $T_B = (1 - F_D)T_A + F_DT_D$. If all of the potassium is carried in the detrital components (mainly sanidine, plagioclase, and possibly basalt glass), $F_D = 1$. In this case, the K-Ar system records a mixture of the ages of components present in the crater rim, principally bedrock ranging from minimally to completely reset by the Gale-forming impact. Crater-rim lithologies, as well as the crater itself, are thought to range from Noachian to Early Hesperian in age or about 4.1 to 3.5 Ga (5, 6, 27–29), in agreement with the K-Ar age of 4.21 ± 0.35 Ga that we measured. Because formation of K-bearing authigenic phases can only lower the mudstone age below that of the detrital component, we conclude with 95% confidence (30) that the potassium-weighted mean age of the materials in the Gale crater wall exceeds 3.6 Ga. Alternatively, if the potassium is entirely hosted within authigenic components (phyllosilicates or amorphous phases other than basalt glass), $F_D = 0$ and the age records the formation age of those phases. In this case, 4.21 Ga would represent a minimum age of mudstone deposition.

At present, we have no definitive way to determine the potassium distribution in the mudstone, but we can make a reasonable estimate. K-bearing phases determined by CheMin include sanidine (1.8%), andesine feldspar (21%), and an unknown fraction of basaltic or impact glass (17). CheMin cannot determine the K_2O content of any of these phases, so we assume representative values of 13%, 0.3%, and 1%, respectively (31). By assuming the mudstone



Fig. 2. Mars Hand Lens Imager (MAHLI) image of brushed, gray bedrock outcrop of Sheepbed mudstone near the Cumberland drill hole. Protrusion of nodules (9) results from eolian scouring of rock surface, creating wind tails. Preference for steep faces of wind tails on northeast side suggests a long-term averaged paleowind direction from northeast to southwest. This is a portion of MAHLI image 0291MH0001970010103390C00, acquired on sol 291. Illumination from the upper left.

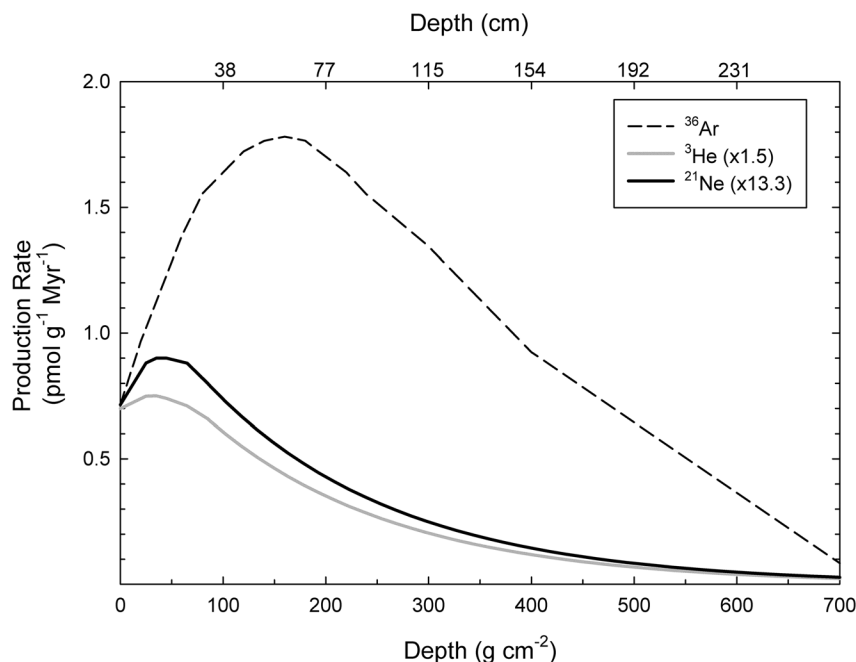


Fig. 3. Depth dependence of cosmogenic isotope-production rates modeled for a rock of Cumberland mudstone chemistry on Mars. Helium-3 and ^{21}Ne are spallation isotopes, whereas ^{36}Ar is produced by capture of cosmogenic neutrons. Note the multiplicative factors applied to ^3He and ^{21}Ne . A mudstone bulk density of 2.6 g cm^{-3} was assumed to convert overburden mass to linear depth.

contains 10% glass, we conclude that the detrital component accounts for 80% of the measured K₂O. The remainder could then be in the authigenic clay (18% of the mudstone) with 0.6% K₂O, in agreement with K₂O concentrations in saponite in a martian meteorite (32). If we assume T_D < 4.1 Ga on the basis of crater-count ages of the Gale impact and host terrains and use the 95% confidence lower limit on the K-Ar age of 3.6 Ga, we obtain a current best estimate that the mudstone was deposited before 1.6 Ga. Assuming either a younger age for the detrital component or a smaller fraction of it in the mudstone would cause this estimate to rise.

Cosmogenic ³⁶Ar, ²¹Ne, and ³He

Isotopes ³⁶Ar, ²¹Ne, and ³He were all detected at levels that cannot be attributed to sources other than cosmic ray irradiation (33). In the mudstone, ³⁶Ar/³He and ³⁶Ar/²¹Ne are 1.7 ± 0.5 and 12 ± 5, respectively (34). These ratios are within error of predictions of the no-erosion scenario (1.5 and 13) and very different from the prediction of the steady-erosion scenario (4 and 30). Thus, we cast our results in terms of surface-exposure age, which for ³He, ²¹Ne, and ³⁶Ar are 72 ± 15, 84 ± 28, and 79 ± 24 Ma, respectively. These ages could be the sum of multiple shorter exposure intervals separated by burial events, for example, by exposure during initial deposition and then again by recent reexposure or alternatively by burial and exhumation associated with migrating eolian deposits. However, these events would have to involve rapid burial and removal of at least a few meters of cover to maintain the good match of measured nuclide concentration ratios to those of production at the surface.

The agreement among these results argues for complete extraction of all three noble gases in the SAM oven and against substantial loss of noble gases over the ~78-million-year (My) period of cosmic ray exposure. For example, diffusive loss of He from amorphous phases, plagioclase, and phyllosilicates might occur at Mars ambient temperature (35), but the ³He/²¹Ne ratio

of ~7.5 ± 2.6 matches the expected production ratio of ~8. Similarly, dissolution and reprecipitation of a water-soluble Cl-rich phase by occasional wetting of the mudstone might release accumulated ³⁶Ar. If this occurred to a substantial extent, then the agreement between the ³⁶Ar exposure age and the ²¹Ne and ³He exposure ages would have to be fortuitous. Although not impossible, we see no evidence for it in these data.

A substantial portion of the ³He and ²¹Ne must be carried by detrital minerals, including plagioclase and pyroxene, because these are important host phases for the major target elements (36). In contrast, the enrichment of Cl compared with a typical martian basalt suggests that much of this element was added to the mudstone from solution (9, 10). This distinction implies that, although ³He and ²¹Ne might record cosmic-ray irradiation that occurred during primary exposure and transport in addition to that acquired during modern exposure at Yellowknife Bay, the same is not true of ³⁶Ar. The logical interpretation of the good agreement among all three exposure ages is that all three reflect exposure only in the modern setting.

The apparent absence of a detrital cosmogenic signal favors deposition of the mudstone in an environment in which erosion and transport were fairly rapid and/or in which the martian surface was shielded from cosmic rays by a reasonably dense atmosphere. The transition from comparatively wet conditions and a thick atmosphere to cold and dry conditions with a thin atmosphere is thought to have occurred around the Noachian-Hesperian boundary (37). Because wet conditions favor rapid erosion, in either scenario the cosmogenic isotope observations would support deposition of the Sheepbed mudstone shortly after Gale formation. Alternatively, deposition may have occurred in association with a later event in which material was eroded and transported rapidly enough to prevent detectable cosmogenic production. A late erosional event has also been suggested for Amazonian-age fan formation in some other martian craters (38–40).

Topography, stratigraphy, and surface-exposure dating suggest that the Yellowknife Bay trough is currently a focus of erosion and that erosion is occurring by scarp retreat. The Sheepbed mudstone lies below a sequence of relatively more-resistant units that define a series of topographic steps (A to A' in fig. S1). The pace of wind erosion for the entire escarpment will be set by the retreat rate of these more-resistant units. However, deflation of the softer units, such as the mudstone, undercuts and contributes to collapse of the resistant layers (Fig. 1), such that both direct wind abrasion of resistant units and removal of the softer units contribute to slope retreat. Resistant units and corresponding local steps will thicken or thin as erosion sweeps into units with variable thicknesses and resistance properties. This predominately lateral erosion has caused scarp migration to the southwest and possibly other directions and produced several meters of surface lowering. The general flattening of the topographic profile (fig. S1) as it extends into the Yellowknife Bay trough may indicate that a more-resistant unit beneath the Sheepbed mudstone has slowed continued removal of this unit.

In such a model, the scarp retreat rate can be estimated from the surface-exposure ages. To prevent cosmogenic nuclide accumulation requires at least 2 to 3 m of overburden. Southwestward from the drill site (and downwind, according to Fig. 3), this amount of overburden is first encountered about 60 m away (fig. S1). Thus, in a model in which the mudstone is rapidly exposed from >2- to 3-m depth to the surface, the scarp retreat rate over the last ~80 My averages ~0.75 m My⁻¹. At this rate, the full extent of the Yellowknife Bay exposure could have been produced over several hundred million years of steady lateral erosion. Alternatively, wind erosion and associated scarp retreat may be highly episodic, perhaps in response to climatic variations tied to Mars' obliquity cycle (41, 42). Either way, surface-exposure ages may vary substantially around the Yellowknife Bay outcrop and other similar exposures, in principle offering a test of the scarp retreat hypothesis.

Implications for Organic Preservation

Cosmic rays penetrating the uppermost several meters of rock create a cascade of atomic and subatomic particles, electrons, and photons that ionize molecules and atoms in their path [e.g., (43, 44)]. Complex organic molecules are particularly susceptible to degradation by such particles, and, because these particles also produce the cosmogenic nuclides, we have a way to assess the likely magnitude of this degradation in the Cumberland sample. Degradation of organic molecules in martian surface rocks subjected to cosmic-ray irradiation has been modeled (44). When scaled to the long-term cosmic-ray dose implied by the cosmogenic nuclides in the Cumberland sample, these rates predict reductions of between 2 and 3 orders of magni-

Table 1. Geochronology data. The elemental composition was obtained from APXS measurement of Cumberland drill tailings from Sheepbed mudstone. PR is the model isotope production rate (47). Surface exposure age assuming no erosion. Indicated uncertainty is one standard deviation.

Cumberland sample mass		0.135 ± 0.018 g				
<i>K-Ar system</i>						
K ₂ O (wt %)	0.50	±0.08				
⁴⁰ Ar (nmol/g)	11.95	±1.71				
K-Ar Age (Ga)	4.21	±0.35				
<i>Cosmogenic isotopes</i>						
Isotope	pmol/g	±	PR (pmol g ⁻¹ Ma ⁻¹)		Exposure age	
			Surface	2-m average	(Ma)	±
³ He	33.7	6.9	0.466	0.171	72	15
²¹ Ne	4.49	1.52	0.054	0.025	84	28
³⁶ Ar	55.6	16.8	0.714	1.029	78	24

tude in the concentration of organic molecules in the 100– to 400–atomic mass unit range.

The scarp retreat hypothesis offers a possible strategy for minimizing the degree of organic degradation in samples obtained during the further course of exploration by Curiosity and possible future missions. Because exposure ages and thus cosmic-ray doses should decrease toward a bounding scarp (at least in the downwind direction), such a location may offer the best potential for organic preservation. Given our estimated scarp retreat rate, locations within a few tens of cm of a few-meter-scale scarp may have been exposed to cosmic rays for less than a few My. Such a short exposure compares favorably to what can reasonably be expected from alternative sampling strategies relying on recent impact craters or drilling to obtain fresh strata.

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Acknowledgments: The authors are indebted to the Mars Science Laboratory (MSL) Project engineering and management teams for their exceptionally skilled and diligent efforts in making the mission as effective as possible and enhancing science operations. We are also grateful to all those MSL team members who participated in tactical and strategic operations. Without the support of both the engineering and science teams, the data presented here could not have been collected. Three anonymous reviewers provided many helpful suggestions. Some of this research was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. Data presented in this paper are archived in the Planetary Data System ([pds.nasa.gov](#)).

Supplementary Materials

[www.sciencemag.org/content/343/6169/1247166/suppl/DC1](#)
Materials and Methods
Figs. S1 to S3
Tables S1 to S4
References (48–67)

14 October 2013; accepted 25 November 2013
Published online 9 December 2013;
[10.1126/science.1247166](#)



Ancient Aqueous Environments at Endeavour Crater, Mars

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Science **343**, (2014);

DOI: 10.1126/science.1248097

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Ancient Aqueous Environments at Endeavour Crater, Mars

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Opportunity has investigated in detail rocks on the rim of the Noachian age Endeavour crater, where orbital spectral reflectance signatures indicate the presence of Fe⁺³-rich smectites. The signatures are associated with fine-grained, layered rocks containing spherules of diagenetic or impact origin. The layered rocks are overlain by breccias, and both units are cut by calcium sulfate veins precipitated from fluids that circulated after the Endeavour impact. Compositional data for fractures in the layered rocks suggest formation of Al-rich smectites by aqueous leaching. Evidence is thus preserved for water-rock interactions before and after the impact, with aqueous environments of slightly acidic to circum-neutral pH that would have been more favorable for prebiotic chemistry and microorganisms than those recorded by younger sulfate-rich rocks at Meridiani Planum.

The Mars Exploration Rover Opportunity has been exploring Endeavour crater, an impact crater ~22 km in diameter formed in ancient Noachian materials, since August 2011 (1). Opportunity arrived at Cape York, an eroded segment of Endeavour's rim, where the rover traversed from the younger Burns formation sulfate-rich sandstones onto the older rim rocks (2) (Fig. 1). The rover initially traversed onto the southern tip of Cape York, named Spirit Point, where impact breccias were detected and characterized (1). After traversing along the western side of Cape York and spending the martian winter near its northern end, Opportunity traversed back south-

ward along the eastern side of Cape York when spring arrived.

Observations from the Mars Reconnaissance Orbiter's (MRO's) Compact Imaging Spectrometer for Mars (CRISM) (3, 4), acquired in an along-track oversampled (ATO) mode to sharpen spatial details, were used to identify and map a Fe⁺³-rich smectite mineral locality in an area on the eastern side of Cape York called Matijevic Hill (5) (Fig. 1). Opportunity and its Athena instrument payload (6, 7) were then used to investigate this area in detail for a total of 200 sols (8) to determine the source of this mineral signature and implications for past environmental conditions (Fig. 1).

CRISM Observations

An extensive ensemble of standard-mode CRISM data with ~18 m/pixel spatial resolution has been acquired over Endeavour crater and its rim segments since 2006 to help identify areas that expose minerals formed in aqueous environments (9–11). CRISM observations using the ATO mode were acquired beginning in 2010 over Cape York to identify and map aqueous minerals in detail so that Opportunity could then be directed to those localities to make ground-based observations of the relevant deposits. The CRISM ATO data were collected by gimballing the instrument optical system to space the normally ~18-m pixels (projected onto the ground) to a few meters apart in the along-track direction. Damped least-squares processing techniques were then used to sharpen the spatial resolution to 9 m/pixel in this direction (supplementary materials). Because Cape York is approximately aligned along the MRO ground track, this approach allowed identification of outcrops at a much finer spatial scale than was possible with previous observations. Using a first-principles approach to model the atmospheric gases, aero-

sols, and surface scattering behavior, CRISM data for each wavelength band were reduced to surface single scattering albedo (SSA), a parameter that is independent of lighting and viewing conditions (supplementary materials). For this study, the retrieved SSAs were recast to spectral radiance coefficients using the lighting and viewing conditions for laboratory data acquired with the Brown University RELAB spectrometer system. This allowed direct spectral feature and magnitude comparisons between RELAB and CRISM data.

Retrieved SSA spectra (0.45 to 2.5 μ m) were examined interactively for all of Cape York and surrounding plains, along with use of standard band depth mapping to search for evidence of clay mineral signatures. Results show that there is one small region, located on the Endeavour crater side of Cape York on Matijevic Hill, that shows 2.28 and 2.39 μ m Fe-OH combination absorptions diagnostic of an Fe⁺³-rich smectite best matched by the mineral nontronite (12–15) (Fig. 2). The locations exhibiting these features were mapped using >1.0% absorption band depth criteria (Fig. 1). The ~1.9- μ m band depth for the average spectrum for this location is indistinguishable from the average spectrum for all of Cape York, which implies a high degree of desiccation of the inter-layer water (16, 17) (supplementary materials). The lack of ~1.4- μ m absorption is also consistent with substantial desiccation. Further, the 2.28- and 2.39- μ m absorptions are shallow, even compared to laboratory-derived spectra of a mix of anhydrous basalt and 5% by weight of Fe⁺³-rich smectite (18, 19) (Fig. 2). This implies that the Fe⁺³-rich smectite on Cape York likely occurs at only a few weight percent of the exposed material.

Opportunity Observations

Overview

Opportunity was commanded to turn uphill and start a detailed investigation of the rocks on Matijevic Hill at the location where CRISM data showed the Fe⁺³-rich smectite signature (Fig. 1). These localities correspond to rocks subsequently named the Matijevic formation that range from light-toned, planar outcrops with a discontinuous surface of darker veneers to erosionally resistant ridges with high concentrations of small spherules (Figs. 3 and 4). In a few locations, apparent bedding exposed in cross section is expressed as millimeter- to centimeter-scale layers (Fig. 5). The Matijevic formation rocks are fine-grained, with subrounded particles ranging from ~0.3 mm in size to below the limit of Microscopic Imager (MI) resolution (supplementary materials). Rare dark particles are present, with shapes that range from angular to subrounded. The energy per volume required to grind into spherule-free exposures of these rocks with the rover's Rock Abrasion Tool (RAT) is ~2.8 J/mm³, similar to soft sulfate-rich sandstones elsewhere at the Opportunity site (20) and to some of the weakest rocks found by the rover Spirit (21).

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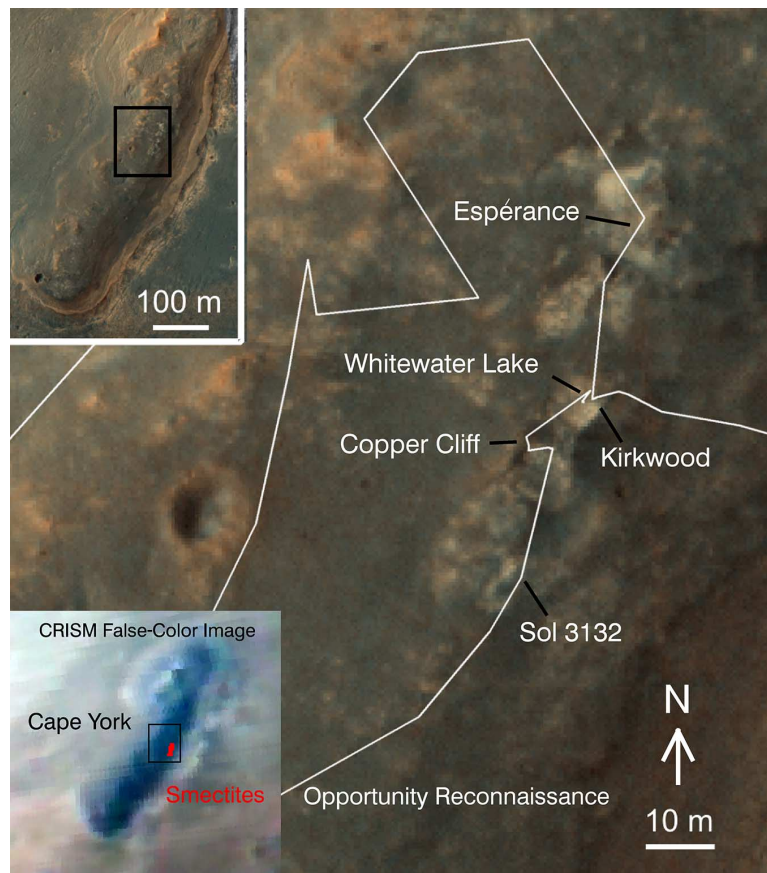
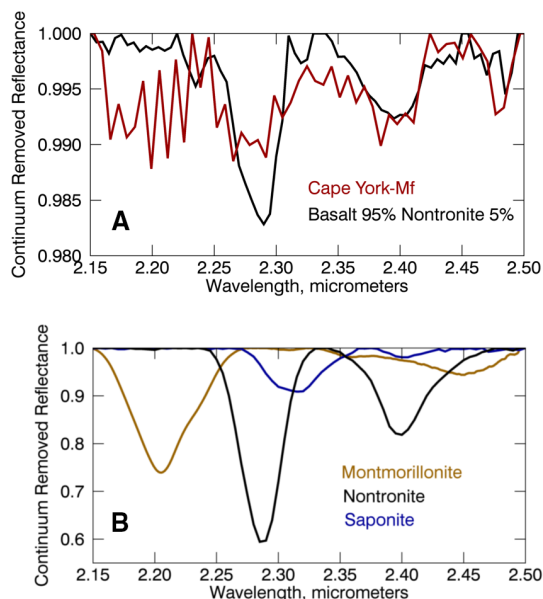


Fig. 1. Inset, top left, is a portion of a false-color HiRISE image centered on Cape York. Black outlines Matijevo Hill as shown in the main figure with initial traverses accomplished by Opportunity to evaluate the geologic setting of the region for which CRISM data showed the presence of Fe^{3+} -rich smectite. Key named locations and the Espérance target are shown, along with the location from which the sol 3132 Navcam mosaic shown in Fig. 3 was taken. Lower left shows a portion of CRISM ATO FRT0001D86B centered on Cape York and processed to 9 m/pixel along track and projecting RGB as 2.2, 1.8, and 1.2 μm (see supplementary materials). The red region in the CRISM insert delineates where CRISM spectra show 2.28- and 2.39- μm absorptions diagnostic of Fe^{3+} -rich smectite. HiRISE observation ESP_032573_1775_color.jp2.

Fig. 2. (A) CRISM-based, continuum-removed mean spectrum for the Fe^{3+} -rich smectite locations on Matijevo Hill, together with a laboratory spectrum for the mix of 95% basalt and 5% nontronite (18, 19). As explained in Supplementary materials, the increased noise level for wavelengths less than ~ 2.2 μm precludes identification of subtle metal-OH absorption features for this wavelength interval. (B) Continuum-removed laboratory spectra of 100% fine-grained (clay-sized) montmorillonite (Al-rich), nontronite (Fe^{3+} -rich), and saponite (Mg-rich) smectites are plotted. Nontronite (12) is the best spectral match to the CRISM spectrum. Saponite spectrum is from sample LASA59 in the RELAB archives at Brown University. Montmorillonite spectrum is from sample SWY-2, documented in (37).



The elemental composition of most Matijevo formation rocks, as determined by the Alpha Particle X-Ray Spectrometer (APXS) (Table 1), is similar to average martian soil (i.e., basaltic) (22), similar to average martian upper crust on a S- and Cl-free basis (23), and slightly higher in Si and Al and lower in Fe than the Shoemaker formation impact breccias that make up most of Cape York (1). Analysis of photon-scattering peaks (24) in APXS data from Matijevo materials does not reveal excess light elements within detection limits. This observation places an upper limit of ~ 5 weight percent (wt %) H_2O in the rock. The inferred lack of hydration is consistent with the CRISM-based Fe^{3+} -rich smectite detection that shows Fe-OH combination bands at 2.28 and 2.39 μm but lacks evidence for 1.4- or 1.9- μm bands indicative of extensive interlayer water (see the supplementary materials).

The Fe^{3+} -rich smectite signature seen in CRISM data likely results from the presence of the veneers because these deposits are enriched in elements (Zn, S, Cl, and Br) that are mobile under aqueous conditions and match the signature from CRISM data maps to locations where veneers are present (Figs. 1 and 3). Panoramic camera (Pancam) spectra show that, where dust has been brushed away using the RAT, the veneers exhibit a subtle absorption centered over the 0.934- μm band (Fig. 6). This feature is consistent with, but not uniquely indicative of, the presence of a Fe^{3+} -rich smectite electronic transition absorption (12). An equivalent feature is not apparent in CRISM spectra retrieved for the CRISM S data (~ 0.45 to 1.0 μm) for the region where the 2.28- and 2.39- μm absorptions are present. This is likely because of the subtle nature of the 0.934- μm absorption and obscuration by wind-blown dust that dominates spectra for these wavelengths in most areas of Mars, including Endeavour's rim segments.

Spherules

The spherules that are present in many Matijevo formation rocks are found in concentrations that range up to $\sim 40\%$ by volume, with highest values at the Kirkwood locality (Fig. 7). Spherules are typically 2 to 3 mm in diameter, with a ~ 5 -mm maximum diameter. Wind erosion of the spherules has exposed concentric structures, with resistant outer shells, less resistant interiors that are visually similar to the surrounding matrices, and irregular and resistant internal structures. Spherules are mostly matrix-supported, even at the Kirkwood locality, although some are in contact with one another. Kirkwood lacks laminar bedding, although roughly horizontal partings accentuated by wind erosion are weakly expressed. Spherule-rich outcrops like Kirkwood are resistant to erosion relative to materials around them and, thus, stand out in positive relief. The RAT specific grind energy of Kirkwood is ~ 23 J/mm³, ~ 8 times that of spherule-free Matijevo formation rocks.

Spherules exhibit subtle compositional differences compared to the matrix in which they

are embedded (Fig. 8). FeO_T increases with increasing spherule abundance, whereas CaO , Al_2O_3 , and MnO decrease. The FeO_T/MnO ratio varies from <50 in spherule-free Matijevic formation materials to >75 in spherule-rich targets, extrapolating to >100 in pure spherules. Spherules are also slightly redder in Pancam color images than the matrix in which they are embedded, and cuttings produced by RAT abrasion of dense accumulations of spherules show a subtle $0.535\text{-}\mu\text{m}$ absorption in Pancam data that

is consistent with the presence of fine-grained iron oxides.

Impact Breccias

Overlying the Matijevic formation is a unit that is best exposed at Copper Cliff (Figs. 1, 3, and 9A). The contact at this location is planar and likely an unconformity, with an abrupt transition from the light-toned, fine-grained, orthogonally jointed Matijevic formation rocks upward into darker rock exposures in Copper Cliff with coarse, poorly

sorted rock clasts and no visible jointing. MI images show Copper Cliff rocks are breccias, with clasts up to a few centimeters in size (Fig. 9B). Some spherules are present and show a decrease in abundance up section.

The elemental composition of the Copper Cliff breccias (Table 1) differs from that of Shoemaker formation impact breccias found elsewhere on Cape York (1), particularly near the bottom of the section on Matijevic Hill. The lowermost target, Onaping, has higher Al_2O_3 and lower FeO_T than

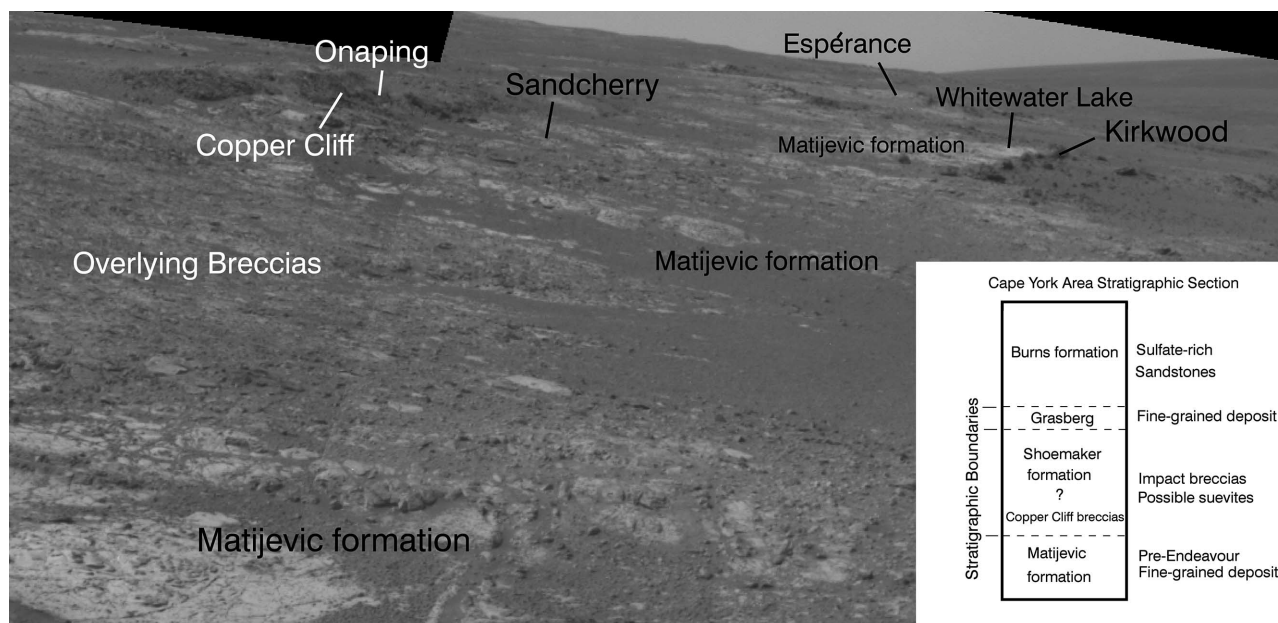
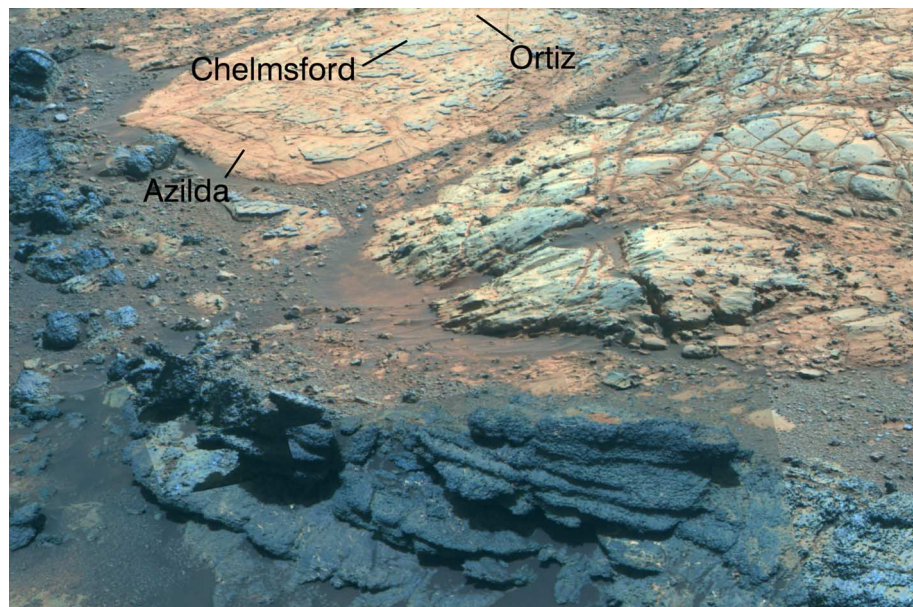


Fig. 3. A portion of a Navcam image mosaic acquired on sol 3132 (see Fig. 1 for the location on Cape York) looking to the north and northeast at recessive, bright, finely layered Matijevic formation outcrops partially covered by dark veneers. Opportunity in situ observations were acquired for the Onaping, Sandcherry, and Espérance targets, in addition to several targets at Whitewater Lake and Kirkwood (see Fig. 4). The Matijevic formation materials are overlain by impact breccias (including rocks exposed on Copper Cliff) on the upslope portion of the scene.

These breccias may or may not be part of the Shoemaker formation breccias exposed over much of Cape York. Matijevic formation outcrops extend to the right and downhill of the scene for ~ 30 m, whereas in the Whitewater Lake and Kirkwood areas, these rocks extend only ~ 4 m along the downslope direction. Stratigraphic section for the local area and surroundings is shown bottom right. The Grasberg unit includes materials that form the bench that surrounds Cape York (1).

Fig. 4. Portion of a Pancam false-color mosaic acquired between sols 3064 to 3070 of the Kirkwood (dark outcrop at bottom of figure) and Whitewater Lake (planar bright outcrops with dark veneers) areas showing the targets Azilda, Chelmsford (vener), and the Ortiz (veins). For scale, the distance between the Azilda and Ortiz targets is ~ 60 cm. Pancam bands L2 ($0.753\text{ }\mu\text{m}$), L5 ($0.535\text{ }\mu\text{m}$), and L7 ($0.432\text{ }\mu\text{m}$) shown as RGB.



Shoemaker formation breccias, whereas other rocks sampled that lie stratigraphically above Onaping (Vermilion Cliffs, Vermilion Lake, and Maley) become increasingly similar to Shoemaker formation breccias. All of these targets have high Ni compared with other Shoemaker formation breccias, but similar to those of the Matije vic formation rocks.

Sulfate-Rich Veins

Both Matije vic formation rock exposures and the overlying Copper Cliff breccias locally contain narrow, light-toned fracture-filling veins (Fig. 10A). Maximum widths are ~1 cm, and most are much

narrower. Multiple APXS measurements on dense concentrations of veins show a strong correlation of CaO with SO₄ (Fig. 10B), in a ratio consistent with a dominance by Ca-sulfate. Pancam multi-spectral images of the veins show a marked downturn in reflectance from 0.934 to 1.009 μm. This same downturn has been observed for much larger Ca-sulfate veins west of Cape York, where it was attributed to a H₂O overtone feature indicative of the hydrated CaSO₄ mineral gypsum (1, 25).

Boxwork Fractures

In a few locations, Matije vic formation outcrops are cut by decimeter-scale boxwork fractures

defined by planar fins and vertical laminae that lie parallel to quasi-orthogonal joint planes. A distinctive linear zone along one of the joint planes, named Espérance, is ~0.5 m long, with an irregular width reaching ~0.1 m. This area was the site of an intensive measurement campaign. The bulk of the material in Espérance is brighter than the host rock, with patchy darker coatings. The chemical composition of Espérance (Table 1) is noteworthy. After partial RAT abrasion of the target Espérance6, APXS data show the lowest values of FeO_T (4.4 wt %) and CaO (2.1 wt %), and the highest values of SiO₂ (62.5 wt %) and Al₂O₃ (15.4 wt %) measured by Opportunity at Meridiani Planum. In addition, Pancam images of the brightest regions of Espérance show a downturn in reflectance from 0.934 to 1.009 μm, consistent with the presence of one or more hydrated mineral phases [e.g., hydrated silica (25)], although APXS scatter-peak ratios constrain the water content to be less than ~5 wt %.

Interpretations

The origin of Matije vic formation rocks is constrained, although not uniquely, by their fine-grained and locally layered character. These rocks have been observed, to date, only on Matije vic Hill, and thus, broader geologic context for their formation is lacking. Fine-grained clastic rocks can form by impact, explosive volcanic, eolian, or fluvial and/or lacustrine processes, and without context, we cannot distinguish confidently among these possibilities. If the deposits are an impactite, the fine-grained nature, with overlying coarse breccias, implies that they are distal ejecta from an impact that predates Endeavour, not from the Endeavour impact itself. Whatever their origin, the Matije vic formation exposes the oldest materials investigated to date by Opportunity.



Fig. 5. Pancam false-color view acquired on sol 3066 of fine-scale layering in the Whitewater Lake locality. Veneers have been resistant to wind erosion and enhanced the layered appearance of the outcrop. Layers are typically several millimeters thick.

Table 1. Elemental chemistry of selected samples as determined by the APXS instrument, under the standard assumption of a homogeneous APXS target matrix. FeO_T denotes total iron oxides. MfLM, Matije vic formation matrix; MfLV, Matije vic formation veneer; MfSR, Matije vic formation spherule-rich; CCB, Copper Cliff breccia; ESP, Espérance.

Sample		Portion (wt %)													Concn. (μg g ⁻¹)		
		Na ₂ O	MgO	Al ₂ O ₃	SiO ₂	P ₂ O ₅	SO ₃	Cl	K ₂ O	CaO	TiO ₂	Cr ₂ O ₃	MnO	FeO _T	Ni	Zn	Br
MfLM	Azilda ¹	2.55	7.91	10.60	51.2	1.50	2.47	0.53	0.28	5.98	0.87	0.24	0.36	15.4	922	134	48
MfLV	Sandcherry ¹	2.83	8.64	9.02	44.7	1.33	6.42	1.75	0.31	7.05	0.86	0.24	0.39	16.3	914	373	332
MfLM	Ortiz (no veins)	2.21	6.58	9.62	46.5	1.23	7.87	0.92	0.32	7.91	0.92	0.23	0.47	15.1	723	193	157
MfLM	Ortiz2b (vein-rich)	2.09	6.28	8.57	42.0	1.17	13.51	0.95	0.27	10.35	0.78	0.22	0.47	13.2	670	144	208
MfSR	Kirkwood ²	2.44	8.47	9.91	49.1	0.74	4.50	1.08	0.49	5.03	0.79	0.30	0.22	16.7	881	134	112
MfSR	Fullerton ²	2.25	8.22	10.47	50.1	0.89	4.64	0.85	0.33	5.81	0.96	0.29	0.28	14.7	738	176	159
MfSR	Sturgeon River ¹	2.21	9.29	9.61	49.5	0.59	3.32	0.47	0.36	5.11	0.81	0.36	0.29	17.9	1165	132	57
CCB	Onaping	2.24	8.21	11.26	47.0	0.99	6.74	1.04	0.27	6.99	0.90	0.28	0.39	13.6	684	212	62
CCB	Vermilion Cliffs	2.25	8.09	10.27	45.0	1.04	8.71	1.27	0.31	7.16	0.83	0.26	0.40	14.2	868	216	312
CCB	Vermilion Lake	1.93	7.28	8.60	44.4	1.14	9.27	1.52	0.50	7.27	1.01	0.29	0.38	16.2	818	600	80
CCB	Maley ²	2.24	8.17	8.94	43.6	0.99	9.79	1.70	0.41	7.02	0.87	0.25	0.36	15.5	863	414	85
ESP	Espérance2	2.16	6.49	10.36	50.6	1.26	8.93	2.61	0.45	5.80	0.99	0.28	0.27	9.6	707	484	233
ESP	Espérance6 ³	2.25	4.73	15.37	62.5	1.14	3.28	2.32	0.24	2.14	0.93	0.34	0.19	4.4	622	238	35
ESP	Lihir	1.66	5.89	12.92	58.4	1.19	6.25	1.58	0.37	4.03	1.16	0.32	0.16	5.8	644	304	114
	Dark soil ⁴	2.34	7.33	9.65	47.0	0.85	4.68	0.59	0.51	7.38	0.90	0.39	0.39	17.6	349	199	24
	Average error	±0.21	±0.11	±0.13	±0.4	±0.08	±0.09	±0.02	±0.06	±0.05	±0.07	±0.03	±0.01	±0.1	±50	±20	±20

¹Sample abraded using the RAT.

²Sample brushed using the RAT.

³Sample partially abraded using the RAT.

⁴Typical Meridiani basaltic sand composition

Fig. 6. (A) Pancam false-color view showing the Chelmsford veneer after brushing using the RAT. Brushed areas are ~3.8 cm wide. Data acquired on sol 3098. **(B)** Pancam-based spectra of undisturbed bright layer, together with Chelmsford veneer undisturbed and brushed surfaces. Note the presence of the subtle absorption associated with the Pancam data acquired on sol 3098.

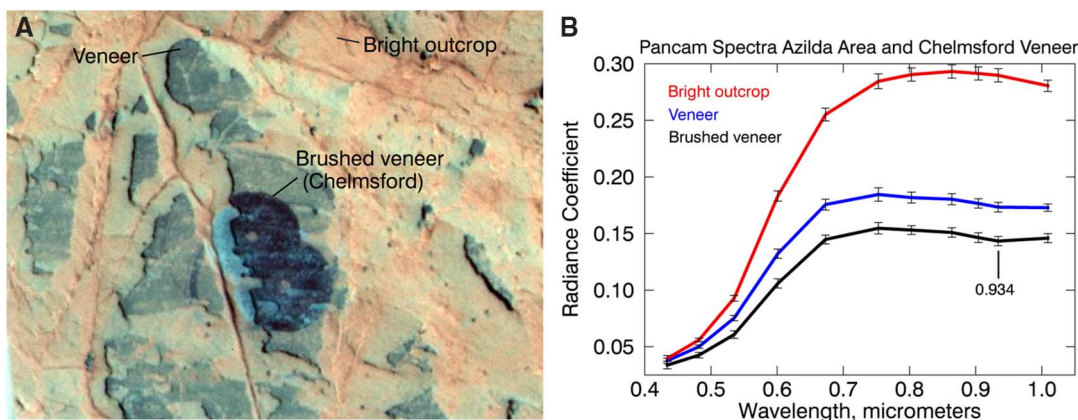
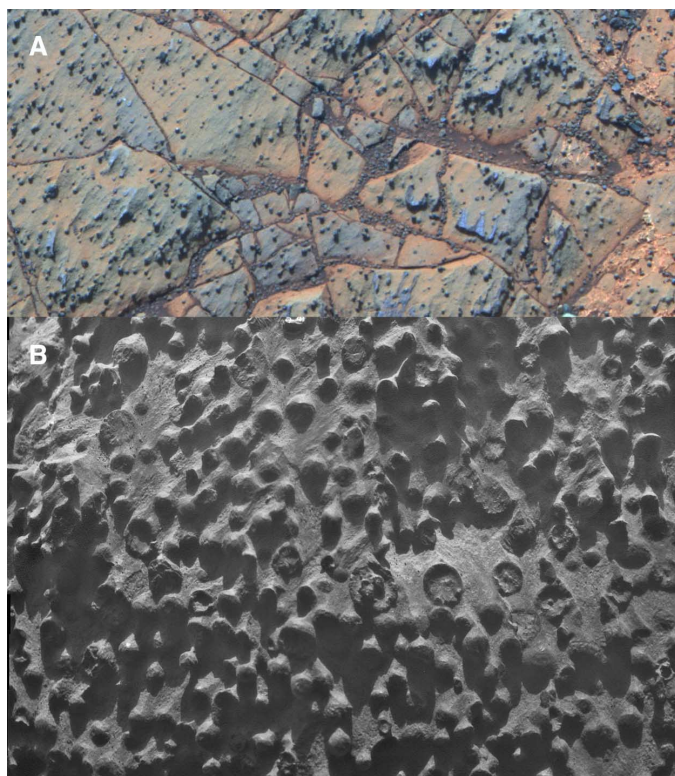


Fig. 7. (A) Pancam false-color image acquired on sol 3208 of Matijevic formation rocks at the White-water Lake locality, showing embedded spherules. Approximate scale across image is 40 cm. **(B)** MI mosaic acquired on sol 3064 showing a dense concentration of spherules at the Kirkwood target and locality. Approximate scale across the scene is 5 cm, and illumination is from the top.



The veneers are likely the carrier of the Fe^{+3} -rich smectite signature detected from CRISM data and are inferred to have formed either as surface deposits or along bedding plane fractures as mildly acidic (>5 pH) (26, 27) waters were neutralized by reactions with the finely layered strata. These produced a small amount of Fe^{+3} -rich smectite and salts.

Two hypotheses are considered for the origin of the spherules: (i) diagenetic concretions and (ii) accretionary lapilli (impact or volcanic). Textural arguments do not by themselves eliminate either hypothesis. Concretions with concentric zoning (i.e., hard exteriors) similar to those observed in Matijevic Hill spherules can form under conditions of diffusion limitation from pore waters characterized by variable pH and low, but variable, oxygen availability (28–30). However, concentric structures are also found in some impact

lapilli (31, 32). Spherules in Matijevic formation rocks are found dispersed through finely layered bedding (Fig. 5). Hydraulic segregation according to particle size (and hence settling velocity) during entrainment by fluids should lead to sorting and deposition of coarse particles before fine particles (33). The observed dispersion of spherules across fine bedding in Matijevic formation rocks therefore favors an origin as concretions. Dense concentrations of spherules are observed at Kirkwood that fall near the high end of concretion densities found on Earth. This would require unusual bed-by-bed variation in rates of fluid flow or availability of nucleation sites, which occasionally does happen on Earth during formation of concretions. Dense decimeter-thick layers of accretionary lapilli are also well documented in impact deposits and occur within a finer-grained matrix (34).

Perhaps the strongest evidence favoring a spherule origin as concretions is the difference in chemistry between the spherules and their matrix, but this difference is subtle (Fig. 8). If the spherules are concretions, they must be lightly cemented as compared to the hematite-rich concretions of the Meridiani plains (2). Extrapolating compositional data for spherule-rich rocks to 100% spherules suggests only about 20% FeO_T (Fig. 8). If the change in FeO_T concentration reflects an increase in Fe^{+3} , then cementation may involve a small proportion of ferric oxide or oxyhydroxide, consistent with the spectral properties of spherule cuttings. The increased FeO_T/MnO in spherule-rich targets is consistent with changing redox and/or pH conditions such that Fe^{+2} in solution oxidized to Fe^{+3} and precipitated as a thin cement, whereas Mn^{+2} continued in solution to precipitate elsewhere. Interpretation of spherules as lapilli would require layer-specific alteration during diagenesis; therefore, both hypotheses invoke groundwater flow within Matijevic formation rocks.

The rocks exposed at Copper Cliff and higher up the side of Matijevic Hill are impact breccias that probably date from the Endeavour impact event. They are either part of or are overlain by Shoemaker formation breccias exposed over much of Cape York (1) and are the stratigraphically lowest breccias examined by Opportunity to date. The spherules they contain may have been released from the Matijevic formation rocks during impact and mixed into the breccias. The different chemical compositions of the lower Copper Cliff breccias, as compared to Shoemaker formation breccias, also suggests some admixture of Matijevic materials, moderate aqueous alteration after emplacement, and/or a differing provenance reflected in a higher feldspathic/mafic ratio in the lithic fragments. The high Ni content suggests Ni mobilization by alteration fluids subsequent to emplacement of the breccias.

The sulfate-rich veins observed in both the Matijevic formation and at Copper Cliff formed when narrow fractures were filled by calcium sulfate precipitated from fluids generated within the underlying Noachian crust. Calcium sulfate was likely precipitated closest to the Noachian

Fig. 8. Oxide concentrations in spherule-bearing targets are plotted as a function of the fraction of the APXS field of view filled by spherules as determined from MI images. Error bars represent 2- σ error for precision of the measurements.

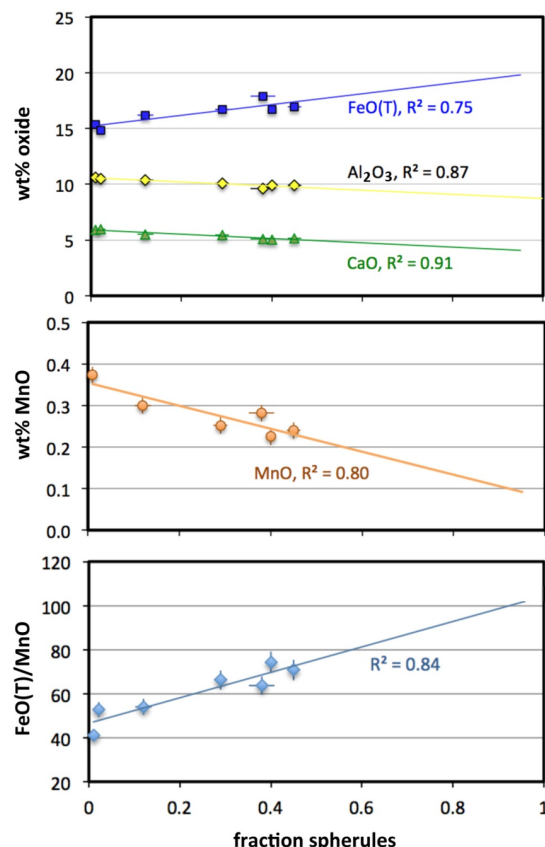
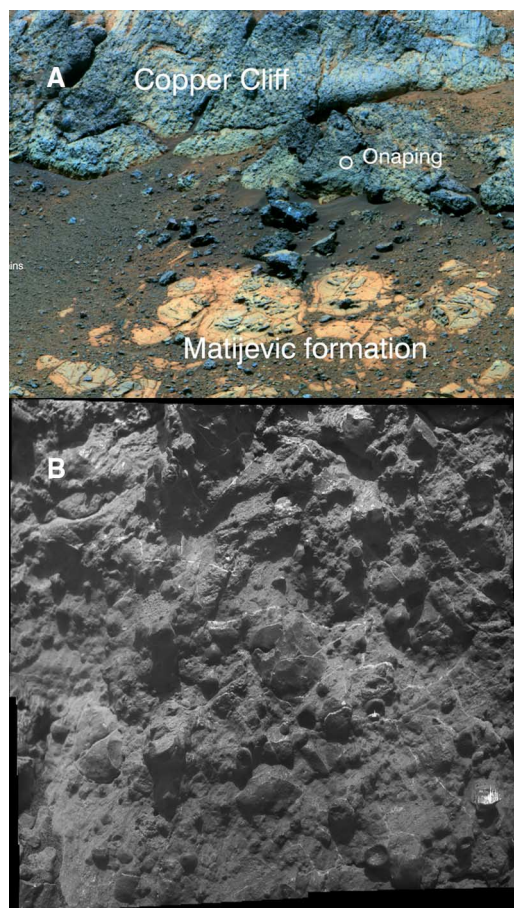


Fig. 9. (A) Portion of a Pancam false-color mosaic acquired over sols 3137-3150, showing dark-toned impact breccias of the Copper Cliff outcrop overlying lighter-toned Matijevec formation materials. The width across the dark outcrops is ~1.5 m. Location of Onaping MI mosaic shown in (B) is indicated by circle. (B) MI mosaic acquired on sol 3158, showing breccia clasts, spherules, and light-toned veins in the target Onaping. Approximate scale across the image is 6 cm, and illumination is from the top.



source rocks, rather than other sulfates (e.g., $\text{MgSO}_4 \cdot n\text{H}_2\text{O}$; $\text{FeSO}_4 \cdot n\text{H}_2\text{O}$) or chlorides, because of its lower solubility in most aqueous fluids. The veins postdate the Copper Cliff breccias, so this aqueous activity postdates the Endeavour impact, which suggests that impact-driven hydrothermal flow could have been a factor. Centimeter-width, linear gypsum veins have also been observed adjacent to Cape York (1), and the narrower and less regular veins of Matijevec Hill could date from the same episode, although no observed geologic relations confirm this.

The distinctive chemistry of Espérance indicates substantial aqueous alteration, particularly when viewed in the classical ternary diagram of mole fraction $\text{Al}_2\text{O}_3 - (\text{CaO} + \text{Na}_2\text{O} + \text{K}_2\text{O}) - (\text{FeO}_T + \text{MgO})$ (Fig. 11B). In this diagram, silicate minerals that plot along and below the feldspar- $(\text{FeO}_T + \text{MgO})$ join are igneous, whereas, above the join, secondary clay minerals dominate (35). Data for spherule-rich rocks plot toward the $\text{FeO}_T + \text{MgO}$ apex, whereas data for veins plot toward the $\text{CaO} + \text{Na}_2\text{O} + \text{K}_2\text{O}$ apex, consistent with compositional inferences discussed in previous paragraphs. Six APXS measurements were made on Espérance, culminating in the target Espérance6 that was partially abraded with the RAT, plus a seventh nearby point called Lihir (Fig. 11A). These data define a near-vertical trend in the diagram that is interpreted as a mixing line between typical Matijevec formation rocks and the underlying altered rock best represented by Espérance6. Results are consistent with a high concentration of an Al-rich smectite. The Espérance data also show a positive correlation between Al_2O_3 and SiO_2 , with excess silica indicated at low Al_2O_3 values (Table 1). A mineral assemblage that includes substantial amounts of Al-rich smectite and a siliceous phase or phases provides compelling evidence for substantial aqueous alteration. In addition, the loss of iron implies that the fluid was reducing because ferric oxides would have been generated under oxidizing conditions at all but very low pH values.

The boxwork fractures at Espérance and elsewhere are similar to the parallel slablike foliations commonly associated with mineral volume changes at uniform depths on rock exteriors and could have presented pathways for fluid flow. The strong localization of alteration along these fractures indicates that the alteration occurred in place, after the fractures formed.

The events recorded at Matijevec Hill imply an aqueous environment different from those that produced the overlying sulfate-rich Burns formation sandstones. Deposition began with fluvial, eolian, distal impact or explosive volcanic emplacement of layered, fine-grained deposits that dominate the Matijevec formation. These materials underwent minor aqueous modification that generated Fe^{+3} -rich smectite and locally more intense alteration by enhanced fluid flow along fractures that generated relatively high concentrations of clay minerals and hydrated silica-rich materials. Breccias were subsequently

Fig. 10. (A) MI mosaic acquired on sol 3189 of the vein-rich location named “Ortiz” at the Whitewater Lake locality, merged with coregistered Pancam enhanced color data. Circles show the fields of view of four APXS measurements. Circle diameters are 3.8 cm. **(B)** CaO versus SO_3 for the APXS placements on Ortiz traquets (multiple measurements were made at some locations). Error bars are relative counting statistics, 2σ .

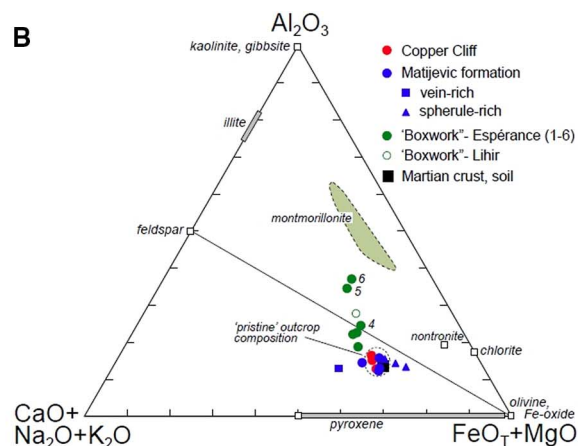
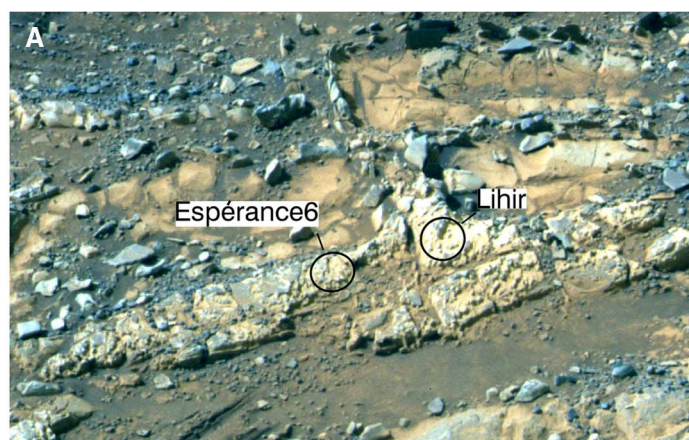
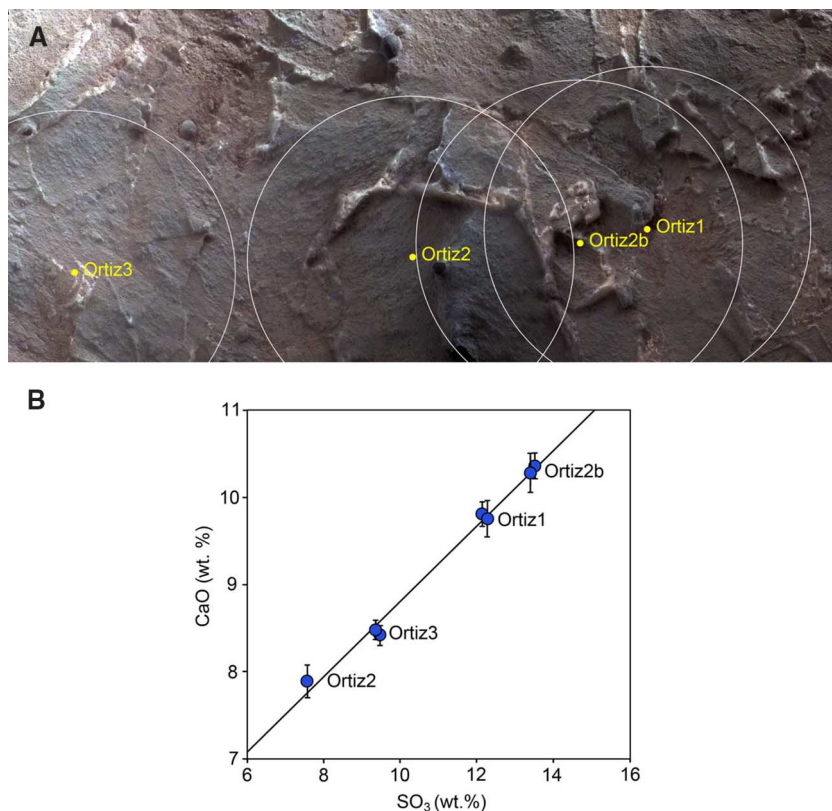


Fig. 11. (A) Pancam false-color view acquired on sol 3230 showing the boxwork structure and examined in detail. Circle indicates the targets Espérance6 (which was abraded with the RAT) and Lihir. Approximate scale across the scene is 70 cm. **(B)** Ternary plot of mole fraction $\text{Al}_2\text{O}_3 - (\text{CaO} + \text{Na}_2\text{O} + \text{K}_2\text{O}) - (\text{FeO}_T + \text{MgO})$

for selected rocks from Matijeic Hill (Table 1) and other materials. Mineral compositions based on idealized stoichiometry, the field for montmorillonite based on structural formulae of 25 natural montmorillonites (38, 39), and average martian crust and soils taken from (23).

emplaced above the Matijeic formation, probably by the Endeavour impact. Subsequently, post-impact fluid circulation, perhaps including impact-triggered hydrothermal flow, led to precipitation of Ca-sulfate veins in fractures that cut through both the Matijeic formation and the overlying Copper Cliff breccias.

The aqueous modification of Matijeic materials provides evidence for the earliest episode of water activity documented by Opportunity. In particular, the unusual chemistry of Espérance points to an early period of localized intense al-

teration under fluid-dominated, near-neutral to modestly low pH and reducing conditions that would, at least transiently, have been more favorable to life or prebiotic chemistry (36) than the very low acidic conditions recorded by younger Burns formation sulfate-rich sandstones.

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12. Nontronite (dioctahedral Fe^{+3} -rich) is one of a family of smectite clay minerals that includes dioctahedral montmorillonite (Al-rich) and trioctahedral saponite (Mg-rich). Nontronite is a common terrestrial weathering product of basaltic rocks. Nontronite spectra are controlled by the presence of Fe^{+3} electronic transition absorptions centered at ~ 0.43 , 0.62 , and 0.93 to 0.94 μm ; an OH stretch overtone absorption at ~ 1.4 μm ; an OH stretch and H-O-H bending mode combination band at ~ 1.9 μm ; and OH stretch and Fe-OH bending mode combination bands at 2.28 and 2.39 μm (13). See (14) for an overview of clay mineral formation and (15) for initial discoveries on Mars.
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Acknowledgments: This work was supported by NASA. The CRISM operations team at the Applied Physics Laboratory, Johns Hopkins University, and the Opportunity operations team at the Jet Propulsion Laboratory, California Institute of Technology were responsible for planning and acquiring the relevant data. The NASA Planetary Data System through the Geosciences Node (<http://pds-geosciences.wustl.edu/>) provides access to the CRISM and Opportunity data used in this paper.

Supplementary Materials
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10.1126/science.1248097

Charge Order Driven by Fermi-Arc Instability in $\text{Bi}_2\text{Sr}_{2-x}\text{La}_x\text{CuO}_{6+\delta}$

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The understanding of the origin of superconductivity in cuprates has been hindered by the apparent diversity of intertwining electronic orders in these materials. We combined resonant x-ray scattering (REXS), scanning-tunneling microscopy (STM), and angle-resolved photoemission spectroscopy (ARPES) to observe a charge order that appears consistently in surface and bulk, and in momentum and real space within one cuprate family, $\text{Bi}_2\text{Sr}_{2-x}\text{La}_x\text{CuO}_{6+\delta}$. The observed wave vectors rule out simple antinodal nesting in the single-particle limit but match well with a phenomenological model of a many-body instability of the Fermi arcs. Combined with earlier observations of electronic order in other cuprate families, these findings suggest the existence of a generic charge-ordered state in underdoped cuprates and uncover its intimate connection to the pseudogap regime.

Since the discovery of cuprate high-temperature superconductors, several unconventional phenomena have been observed in the region of the phase diagram located between the strongly localized Mott insulator at zero doping and the itinerant Fermi-liquid state that emerges beyond optimal doping ($I-20$). The so-called pseudogap (PG) opens at the temperature T^* and obliterates the Fermi surface at the antinodes (ANs) of the d-wave superconducting gap function, leaving behind disconnected “Fermi arcs” centered around the nodes. In addition, charge order has been observed on the surface of Bi- and La-based compounds ($4-8$), in the bulk of La-based compounds ($9-11$), and most recently in $\text{YBa}_2\text{Cu}_3\text{O}_{6+\delta}$ (YBCO) ($17-20$), indicating that this might be the leading instability in underdoped cuprates. The similarity between the observed charge ordering wavevector and the antinodal nesting vector of the high-temperature Fermi surface has prompted suggestions that a conventional Peierls-like charge-density wave (CDW) might be responsible for the opening of the pseudogap ($7, 8, 12, 19$). We used complementary bulk and surface techniques to examine the validity of this scenario and explored the connection between charge ordering and fermiology.

By applying a suite of complementary tools to a single cuprate material, $\text{Bi}_2\text{Sr}_{2-x}\text{La}_x\text{CuO}_{6+\delta}$

(Bi2201), we reveal that the charge order in this system emerges just below T^* , with a characteristic wave vector corresponding to the Fermi arc tips rather than the antinodal nesting vector. We

quantified the Fermi surface by using angle-resolved photoemission spectroscopy (ARPES), and we looked for charge modulations along the Cu-O bond directions in both real and reciprocal space by using scanning-tunneling microscopy (STM) and resonant x-ray scattering (REXS). The single-layer Bi2201 is well suited to this purpose owing to (i) its two-dimensionality and high degree of crystallinity ($21, 22$) and (ii) the possibility of probing the temperature evolution across T^* , which is better characterized ($15, 16$) and more accessible than in bilayer systems. This study, by bringing together three different techniques on the same material belonging to the Bi family together with related observations on La- and Y-based compounds, suggests the ubiquity of charge ordering in underdoped cuprates [see also the related report for bilayer Bi2212 (23)].

REXS uses x-ray photons to exchange momentum with the electrons and the ionic lattice in order to gain information on the electronic charge distribution. As opposed to conventional x-ray diffraction, which is widely used for structural studies, in REXS the photon energy is tuned to resonance with one of the element-specific absorption lines. This results in a strong enhancement of the sensitivity to the valence

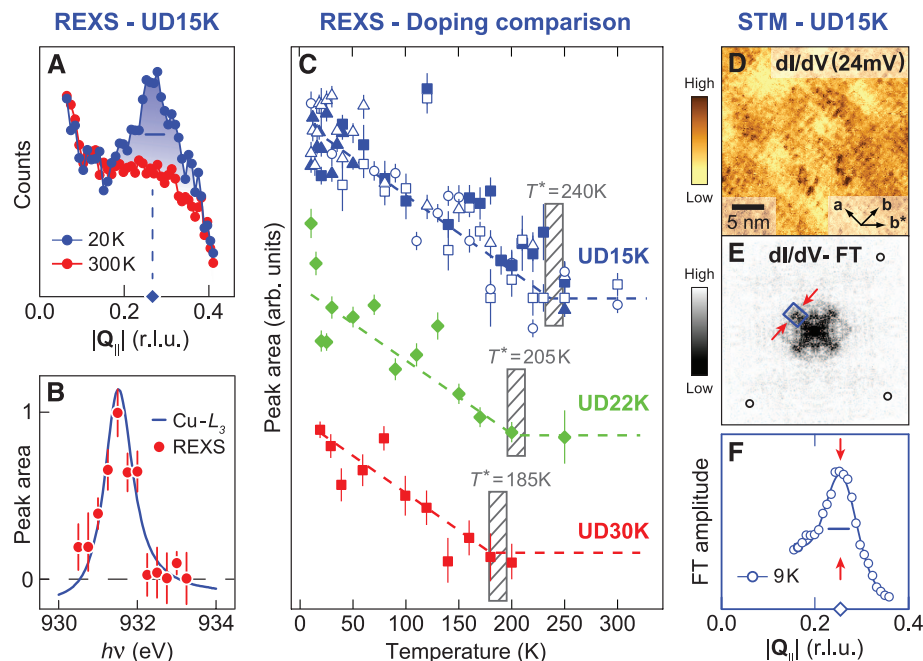


Fig. 1. REXS and STM comparison on Bi2201. (A) Bi2201 low- and high-temperature scans of the in-plane momentum $Q_{||}$, showing the emergence of a CO peak around $Q_{CO} \approx 0.265$. (B) Resonance profile at Q_{CO} after background subtraction (red markers), superimposed onto the Cu-L_3 absorption edge (blue line). (C) Temperature dependence of the CO-peak area for the three Bi2201 doping levels investigated; dashed lines are linear fits, with baseline marking the zero intensity position for each data set. Hollow and full markers for UD15K correspond to positive and negative wave vectors $Q_{||} > 0$ and $Q_{||} < 0$, respectively, for different samples. The PG temperature T^* (gray boxes) is from Knight shift measurements (36). (D) Bi2201 dI/dV map, taken at 24-mV bias over a 29-nm region (9 K, -200 mV, and 250 pA). A charge modulation with period $\sim 4a_0$ is seen in real space. (E) Fourier transform (FT) of (D), after fourfold symmetrization [black circles mark the position of the Bragg vectors $(\pm 1, 0)$ and $(0, \pm 1)$]. Highlighted by the blue box is the region corresponding to the line cut in (F), whose peak structure is suggestive of a periodic modulation with wave vector $Q_{CO} \approx 0.248$. Horizontal bars represent half widths at half maximum.

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electrons and allows the detection of very small variations in the electronic density profile within the CuO_2 planes (II), which are difficult to determine by using nonresonant methods. We performed REXS measurements along the tetragonal crystallographic $\mathbf{a}$ and $\mathbf{b}$ axes, with the corresponding reciprocal axes labeled $\mathbf{Q}_H$ and $\mathbf{Q}_K$, for three doping levels of Bi2201 (24). Because of the near-equivalence of $\mathbf{Q}_H$ and $\mathbf{Q}_K$, we will hereafter use the common notation $\mathbf{Q}_{\parallel}$ and define reciprocal lattice units (r.l.u.) for momentum axes as $2\pi/a_0 = 2\pi/b_0 = 1$, with $a_0 \approx b_0 \approx 3.86 \text{ \AA}$. Figure 1A shows REXS scans at high (300 K) and low (20 K) temperatures acquired on a UD15K sample near the Cu-L_3 absorption peak at a photon energy $h\nu = 931.5 \text{ eV}$. An enhancement of scattering intensity, in the form of a broad peak, is visible at 20 K at $|\mathbf{Q}_{\parallel}| = 0.265 \pm 0.01$, whereas at 300 K it disappears into the featureless background (dominated by fluorescence). By subtracting the latter, we can study the dependence of the low-temperature feature on photon energy, which reveals its resonant behavior at the Cu-L_3 edge (Fig. 1B). The resonant enhancement, together with the absence

of features at the La-M_5 absorption edge (fig. S6), demonstrates that the peak originates from charge order (CO) occurring in the CuO_2 planes. Furthermore, the gradual dependence of the peak intensity on the out-of-plane component of the wavevector $Q_{\perp}$ is similar to observations in YBCO (7) and indicative of short coherence along the $\mathbf{c}$ axis. Figure 1C shows the temperature evolution of the CO peak in REXS: There is an onset temperature T_{CO} , but we cannot conclusively determine whether T_{CO} corresponds to a sharp phase boundary. Although the charge modulation breaks translational symmetry, the system lacks long-range order as evidenced by the short correlation length ($\xi_{\text{CO}} \sim 20$ to $30 \text{ \AA}$). The latter evolves only weakly with doping and temperature (fig. S5) and therefore suggests either strong disorder or substantial fluctuations persisting down to low temperatures (25). In either case, the convergence of T_{CO} and T^* for all doping levels suggests an intimate relationship between the CO and the PG correlations.

STM is used to detect the charge distribution in real space, by scanning an atomically sharp tip over the cleaved Bi2201 surface and map-

ping the differential tunneling conductance $dI/dV(\mathbf{r}, V)$ (where I is current, V is potential, and $\mathbf{r}$ is position), which is proportional to the local density of states at energy $\epsilon = eV$. Here we apply STM to the same UD15K sample studied by REXS. The map of $dI/dV(\mathbf{r}, V = 24 \text{ mV})$ in Fig. 1D shows an incommensurate charge modulation along the $\mathbf{a}$ and $\mathbf{b}$ axes, consistent with either a disordered checkerboard or stripe modulation (25). The Fourier transform of $dI/dV(\mathbf{r}, V)$ (Fig. 1E) and associated line cut (Fig. 1F) quantify the CO peak at $|\mathbf{Q}_{\parallel}| = 0.248 \pm 0.01$. This is in good agreement with Q_{CO} from REXS and also with Q_{CO} recently reported in the context of phonon anomalies in Bi2201 (26). Furthermore, the feature found in STM has a correlation length $\xi_{\text{CO}} \sim 28 \text{ \AA}$, again in agreement with REXS. A summary of the REXS and STM results is presented in Table 1. We therefore arrive at the empirical convergence of a CO that onsets right below T^* (REXS) and whose wave vector is consistent on surface (STM) and bulk (REXS).

The next step is to link the universal surface and bulk charge order to the fermiology. We quantified and clarified this connection by using ARPES to map the Fermi surface on the same UD15K Bi2201 sample studied by REXS and STM (21, 22). In a similar context, the ARPES-derived octet model in the interpretation of quasi-particle scattering as detected by STM (27) is a successful example of such a connection and demonstrates the importance of low-energy particle-hole scattering processes across the “pseudogapped” Fermi surface.

From the raw ARPES data (Fig. 2C) (21), we deduce that the charge-ordering wave vector connects the Fermi arc tips, not the antinodal Fermi surface sections, as had been assumed previously (7, 12, 19). To better understand the empirical link between charge order and fermiology, we first derived the noninteracting band structure by fitting the ARPES-measured spectral function $A_{\text{exp}}(\mathbf{k}, \omega)$ (where $\mathbf{k}$ and ω are electron momentum and energy, respectively) to a tight-binding model (21, 22, 24). The corresponding Fermi surface is shown in Fig. 2A for hole doping $p = 0.12$, equivalent to UD15K (28). The AN nesting, marked by the white arrow, yields an ordering wave vector $Q_{\text{AN}} \sim 0.139$, in disagreement with the REXS/STM average value $Q_{\text{CO}} \sim 0.256$. To account for the suppression of antinodal zero-energy quasi-particle excitations, a hallmark of the PG fermiology, we constructed a model spectral function $A_{\text{PG}}(\mathbf{k}, \omega)$ with an appropriate self-energy $\Sigma_{\text{PG}}(\mathbf{k}, \omega)$, which combines the features found from exact diagonalization of the Hubbard model (29) with the doping-dependent parameters introduced in (30) (see supplementary note 3 for more details). Figure 2B shows how the noninteracting Fermi surface is transformed by the action of our $\Sigma_{\text{PG}}(\mathbf{k}, \omega)$ and also highlights the concurrent shift in the smallest- Q zero-energy particle-hole excitation (gold connectors). The interacting

Table 1. Comparative summary for the CO peak parameters. Data from REXS and STM for the various doping levels. For ARPES, the value listed here corresponds to the observed Q_{HS} , also shown in Fig. 3C. The PG temperature T^* (gray boxes in Fig. 1C) is from Knight shift measurements (36). n/a, not applicable.

Technique	Sample	Parameters			
		Q_{CO} (r.l.u.)	ξ_{CO} ($\text{\AA}$)	T_{CO} (K)	T^* (K)
REXS	UD30K ($p \approx 0.145$)	0.243 ± 0.01	21 ± 3	180 ± 30	185 ± 10
	UD22K ($p \approx 0.130$)	0.257 ± 0.01	23 ± 3	202 ± 20	205 ± 10
	UD15K ($p \approx 0.115$)	0.265 ± 0.01	26 ± 3	237 ± 10	240 ± 10
STM	UD15K ($p \approx 0.115$)	0.248 ± 0.01	28 ± 2	n/a	240 ± 10
ARPES	UD15K ($p \approx 0.115$)	0.255 ± 0.01	n/a	n/a	240 ± 10

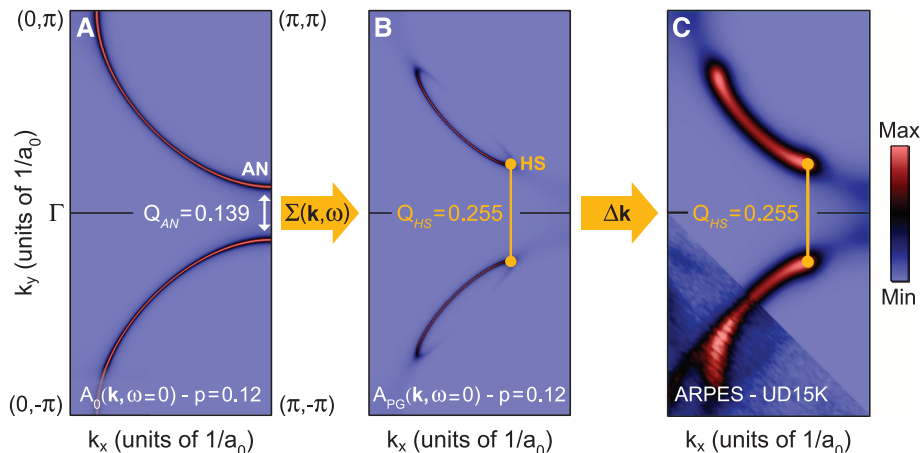


Fig. 2. ARPES and theory comparison on Bi2201. Modeled Fermi surface for hole-doping $p = 0.12$ for (A) the noninteracting and (B) the interacting case, which is computed via the inclusion of the self-energy $\Sigma_{\text{PG}}(\mathbf{k}, \omega)$. A further Gaussian smearing (C), with $\Delta k_x = \Delta k_y = 0.03 \pi/a$ representing the effective experimental resolution, allows comparison between the calculated and measured Fermi surface from UD15K Bi2201 (21). The AN nesting at Q_{AN} (white arrow) can be contrasted with the Q_{HS} -vector associated with the tips of the Fermi arcs (HS), marked by the gold connector.

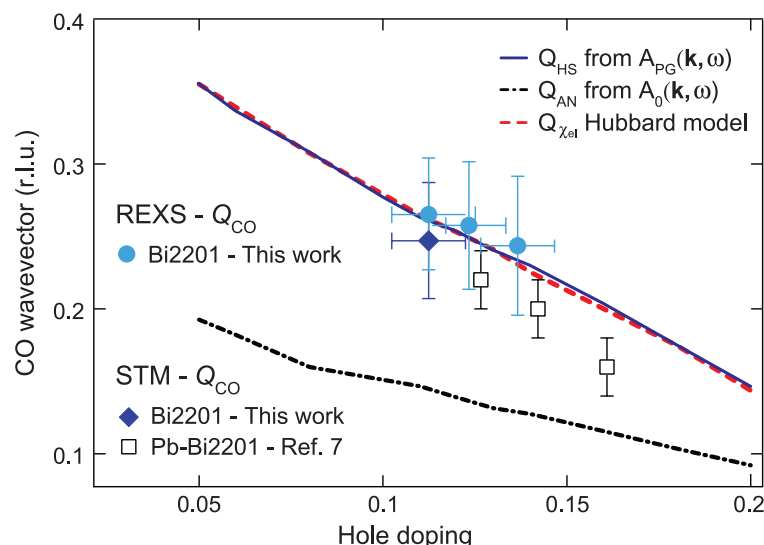


Fig. 3. Doping dependence of the CO wave vector Q_{CO} . Data from REXS and STM on Bi2201 [this work and (7)]; bars represent peak widths rather than errors. Also shown are the doping dependence of the Fermi surface-derived wave vectors Q_{AN} (AN nesting) and Q_{HS} (arc tips) measured from the ARPES spectral function $A_{PG}(\mathbf{k}, \omega)$, as well as the wave vector $Q_{\chi_{el}}$ from the Hubbard-model-based electronic susceptibility (29).

spectral function $A_{PG}(\mathbf{k}, \omega)$ used here is tuned to optimize the match with the corresponding ARPES data (21, 22); after accounting for instrumental resolution, $\Delta\mathbf{k}$, the agreement with the experimental data is excellent, as shown in Fig. 2C. The vector connecting the tips of the Fermi arcs, called hot spots (HSs), is found to be $Q_{HS} \sim 0.255$, closely matching the experimental values of Q_{CO} found for the UD15K sample (see also Table 1).

In Fig. 3, we report the doping dependence of the charge-order wave vector Q_{CO} as seen experimentally, as well as Q_{AN} and Q_{HS} as obtained from the spectral functions $A_0(\mathbf{k}, \omega)$ and $A_{PG}(\mathbf{k}, \omega)$ for the noninteracting and interacting cases, respectively. The superconducting temperature-to-doping conversion for the experimental points is taken from previous studies on La-substituted Bi2201 (28); for Pb-substituted Bi2201 (7), this correspondence might be altered because Pb may contribute holes as well. The mechanism based on electron-hole scattering between AN excitations, with wave vector Q_{AN} , proves to be inadequate throughout the whole doping range. On the other hand, both the wave vector magnitude Q_{HS} and doping-dependent slope dQ_{HS}/dp agree with the Bi2201 experimental data, thereby establishing a direct connection between charge order and HS scattering. To gain further phenomenological insights into a possible link between the ordering of the electronic density and the available charge dynamics, we evaluated the momentum-dependent electronic response (susceptibility) near the Fermi surface, or $\chi_{el}(\mathbf{Q}, \Omega)$ (see supplementary note 3 for more details). We approximate $\chi_{el}(\mathbf{Q}, \Omega)$ as a self-convolution of the single-particle Green's function $\mathcal{G}(\mathbf{k}, \omega)$, in line with a similar approach

successfully used in the study of magnetic excitations in cuprates (31–33). Despite the simplicity of our model, the results for $\text{Re}\{\chi_{el}\}$ along the direction of the experimental charge-ordering confirm that there is an enhancement of particle-hole scattering at a wave vector $Q_{\chi_{el}}$ closely following Q_{HS} (dashed red line in Fig. 3). This convergence supports the idea that accounting for the empirical role played by the HSs is of critical importance for future, more quantitative studies of the electronic instability.

The convergence between the real- and reciprocal-space techniques in our study indicates a well-defined length scale and coherence associated with the electronically ordered ground state. These findings on Bi2201 suggest that the short-ranged charge correlations in Bi-based cuprates (4–7), and the longer-ranged modulations seen in Y-based (18, 19, 34, 35) and La-based compounds (9–11), are simply different manifestations of a generic charge-ordered state [see (23) for related findings on Bi2212]. That the experimental ordering wave vectors can be reproduced through the correlation-induced Fermi arcs in the PG state demonstrates a quantitative link between the single-particle fermiology and the collective response of the electron density in the underdoped cuprates.

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Acknowledgments: We acknowledge S. A. Kivelson, A.-M. Tremblay, S. Sachdev, E. H. da Silva Neto, and A. Yazdani for discussions. This work was supported by the Max Planck–UBC Centre for Quantum Materials, the Killam, Alfred P. Sloan, Alexander von Humboldt, and National Sciences and Engineering Research Council (NSERC) Steacie Memorial Fellowships (A.D.), the Canada Research Chairs Program (A.D., G.A.S.), NSERC, Canada Foundation for Innovation (CFI), and Canadian Institute for Advanced Research Quantum Materials. J.E.H. acknowledges support from the U.S. NSF grant DMR-0847433. A.S. was funded by the A*STAR fellowship. M.M.Y. was funded by an NSERC fellowship. Part of the research described in this paper was performed at the Canadian Light Source, which is funded by the CFI, NSERC, National Research Council, Canadian Institute for Health Research, the Government of Saskatchewan, WD Canada, and the University of Saskatchewan.

Supplementary Materials

www.sciencemag.org/content/343/6169/390/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S10
References (37–46)

10 July 2013; accepted 2 December 2013
Published online 19 December 2013;
10.1126/science.1242996

Ubiquitous Interplay Between Charge Ordering and High-Temperature Superconductivity in Cuprates

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Besides superconductivity, copper-oxide high-temperature superconductors are susceptible to other types of ordering. We used scanning tunneling microscopy and resonant elastic x-ray scattering measurements to establish the formation of charge ordering in the high-temperature superconductor $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+x}$. Depending on the hole concentration, the charge ordering in this system occurs with the same period as those found in Y-based or La-based cuprates and displays the analogous competition with superconductivity. These results indicate the similarity of charge organization competing with superconductivity across different families of cuprates. We observed this charge ordering to leave a distinct electron-hole asymmetric signature (and a broad resonance centered at +20 milli-electron volts) in spectroscopic measurements, indicating that it is likely related to the organization of holes in a doped Mott insulator.

Understanding the mechanism of superconductivity and its interplay with other possible spin or charge organizations in high-transition temperature (T_c) cuprate superconductors remains one of the greatest challenges in condensed matter physics. The observation in La-based cuprates of a suppression of T_c near $x = 1/8$ doping (hole/Cu) coinciding with the organization of charge into stripe-like patterns with four lattice constants (4a) periodicity provided early evidence that charge ordering (CO) competes with superconductivity (1, 2). Recently, x-ray scattering experiments on highly ordered Y-based cuprates have discovered that in the same range of hole concentration (near $x = 1/8$), there is a competing charge organization with an incommensurate ordering vector ($\approx 3.3a$ periodicity) (3, 4). These experiments raise the question of whether there is a universal CO mechanism common to all underdoped cuprates [although crystalline structures in each compound may modify details of the resulting ordering patterns (5, 6)]. If there is a connection between the observed COs in different compounds, what is the mechanism by which this phenomenon competes

or is intertwined with high- T_c superconductivity (7)? Do strong correlations, such as those present as a result of proximity to the Mott insulator, play a role in the CO state and its interplay with superconductivity?

To address these questions, we have carried out scanning tunneling microscopy (STM) and spectroscopy, as well as resonant elastic x-ray scattering (REXS) measurements, on underdoped $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_{8+x}$ (Bi-2212) samples. The Bi-2212 system is the only material system among the cuprates for which there is detailed spectroscopic information on the Fermi surface and the occupied electronic states as a function of temperature and doping, obtained from angle-resolved photoemission spectroscopy (ARPES) performed over the past two decades (8–10). Yet, ARPES measurements have not shown any evidence of band folding associated with CO along the Cu-O bond direction in this system (9, 11, 12). STM studies have long reported evidence of spatial modulation of electronic states in Bi-based cuprates (13–19), but evidence for CO in these experiments is often obfuscated by the disorder-induced energy-dispersive quasiparticle interference (QPI) effect (19–21). The analysis of STM conductance maps over a range of energies has been used to provide evidence for fluctuating charge organization in Bi-2212 below T^* , with strongest enhancement near 1/8 doping—the same doping range in which charge organizations have been seen in Y- and La-based cuprates (19). However, it remains a challenge to clearly separate signatures of CO from those of QPI in STM experiments and, more importantly, to corroborate the surface-sensitive measurements with bulk-sensitive experiments. Here, we provide high-resolution energy-resolved STM spectroscopy experiments that clearly distinguish between QPI and CO features as a function of doping and temperature; this CO signature is also detected in our temperature-dependent REXS measurements.

The geometry of the combined experimental approach for examining CO in underdoped Bi-2212 samples is shown in Fig. 1A, in which we contrast the discrete Fourier transform of STM conductance maps with bulk scattering results from REXS. A typical example of a STM conductance map is shown in Fig. 1B for a UD45 (underdoped Bi-2212, $T_c = 45$ K) sample at 30 K. The discrete Fourier transform of the conductance map (Fig. 1, C and D) shows a strong peak of wave vector $\mathbf{q} = (\pm\delta, 0)2\pi/a$ and $(0, \pm\delta)2\pi/b$, and $\delta \approx 0.3$, along the Cu-O bond directions, corresponding to the real space modulations in Fig. 1B. The results from REXS measurements (incident photon energy of 931.5 eV, resonant with the Cu L_3 -edge) on the same sample are shown in Fig. 1, E and F, as a function of the component of momentum transfer along the a direction (Fig. 1A) (22). The low-temperature REXS measurement (Fig. 1E) shows a peak at the same incommensurate wave vector ($\delta \approx 0.3$) as those in the discrete Fourier transform of the STM conductance maps (Fig. 1, C and D), establishing that the STM modulations on the surface of Bi-2212 sample are in fact due to a CO that can also be detected in the bulk [a similar finding on Bi-2201 is provided in (23)].

To understand why such a CO phenomenon has remained undetected in ARPES studies and to reveal a key spectroscopic characteristic of this ordering, we examined the energy dependence of STM conductance maps. The energy evolution of the features in the discrete Fourier transform of the conductance maps is shown in Fig. 2, along the Cu-O bond direction, measured at 30 K for a range of doping, from optimally doped (OP91) to strongly underdoped (UD15) samples. In the optimally doped sample (Fig. 2A) at temperatures well below T_c , the energy-wave vector structure along the Cu-O bond direction shows no sign of CO. Instead, it shows an energy-dispersing particle-hole symmetric wave vector originating from disorder-induced scattering interference of superconducting Bogoliubov-de Gennes quasiparticles (BdG-QPI) (13, 18, 19, 24). Reducing the doping toward the underdoped regime (Fig. 2, A to G), we found that the BdG-QPI features are systematically weakened, whereas a separate nondispersive modulation with a relatively sharp wave vector appears and strengthens peaking near UD35 (Fig. 2F). This wave vector (for example, $\delta = 0.3$ in Fig. 2, E to G) corresponds to the CO wave vector we find in the REXS measurements (Fig. 1E and fig. S7). The widths in momentum of this CO peak [full width at half maximum, $2\Gamma \approx 0.04$ reciprocal lattice units (rlu)], which agree well with our REXS measurements ($2\Gamma \approx 0.05$ rlu, or equivalently 20a in real space), are larger than those recently observed in the Y-based cuprates (3) and indicate a rather short-range order. Our energy-resolved STM conductance maps further show that these CO modulations only appear over a range of energies (0 to 50 meV) above the chemical potential, a behavior not expected in a conventional

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charge-density-wave (CDW) order. Despite being broad in energy, we found the intensity of the CO feature to be centered at ~ 20 meV above the chemical potential at all doping levels (Fig. 2H). The particle-hole asymmetry of the CO feature in the STM data clearly distinguishes it from the BdG-QPI signals and explains the absence of such CO features in the ARPES studies.

The CO wave vectors extracted from our STM measurements show distinct similarities to other families of the cuprates. For a hole concentration of $x < 0.1$, the incommensurate CO wave vector $\delta \approx 0.3$ matches the CDW observed in the Y-based cuprates, whereas for $x > 0.1$, the nearly commensurate wave vector $\delta \approx 0.25$ is very similar to that found in the stripe phase of La-based cuprates (Fig. 2H). Although an incommensurate δ and its decrease with doping are expected from a Fermi surface nesting mechanism, the narrow doping range over which the jump in δ occurs in Bi-2212 may be an indication of possible competition between two different stable forms of CO in the cuprates. More broadly, though, the fact that CO in Bi-2212, in a range of doping without any structural distortion, can be either similar to Y- or La-based cuprates demonstrates our key point that CO is a ubiquitous phenomenon to underdoped cuprates.

Further evidence that connects measurements on Bi-2212 to other cuprates and probes the interplay between CO and superconductivity comes from examining the temperature dependence of the REXS and STM data. The CO signature in our REXS measurements is first detected at relatively high temperatures (Fig. 1F). By lowering the temperature, the intensity of the CO increases only down to T_c , below which it gradually weakens, suggesting a competition with superconductivity. More detailed signatures of the interplay between CO and superconductivity are observed in our STM measurements as a function of temperature for a UD75 sample (in which temperatures much lower than T_c can be accessed in our experiments). The CO signature above the chemical potential (near $\delta \approx 0.25$ for this sample) is very strong at temperatures just above T_c (Fig. 3A), while being nearly absent when the sample is cooled to roughly $0.15T_c$ (Fig. 3D). Instead, at low temperatures, as shown in Fig. 3D, the STM data for this weakly underdoped sample recover the particle-hole-symmetric dispersing features that are due to BdG-QPI, which is consistent with a d-wave superconducting gap (25). This trend is analogous to the doping-dependent STM data of Fig. 2, in which the CO (measured at a constant temperature, $T = 30$ K) peaks at the UD35 sample (where $T \approx T_c$). Together, these findings not only clearly show superconductivity wins over CO at low temperatures but also provide key information about this competition in momentum space.

Previous analyses of impurity-induced QPI data at low temperatures, such as the data displayed in Fig. 3D, associated the observed dispersing wavevectors along the Cu-O bond direction with

the scattering of BdG quasiparticles between points on the Fermi surface. A schematic Fermi surface for a Bi-2212 UD75 sample is shown in

Fig. 3E together with the different QPI wave vectors connecting different segments of the Fermi surface, starting from near the antinodes at large

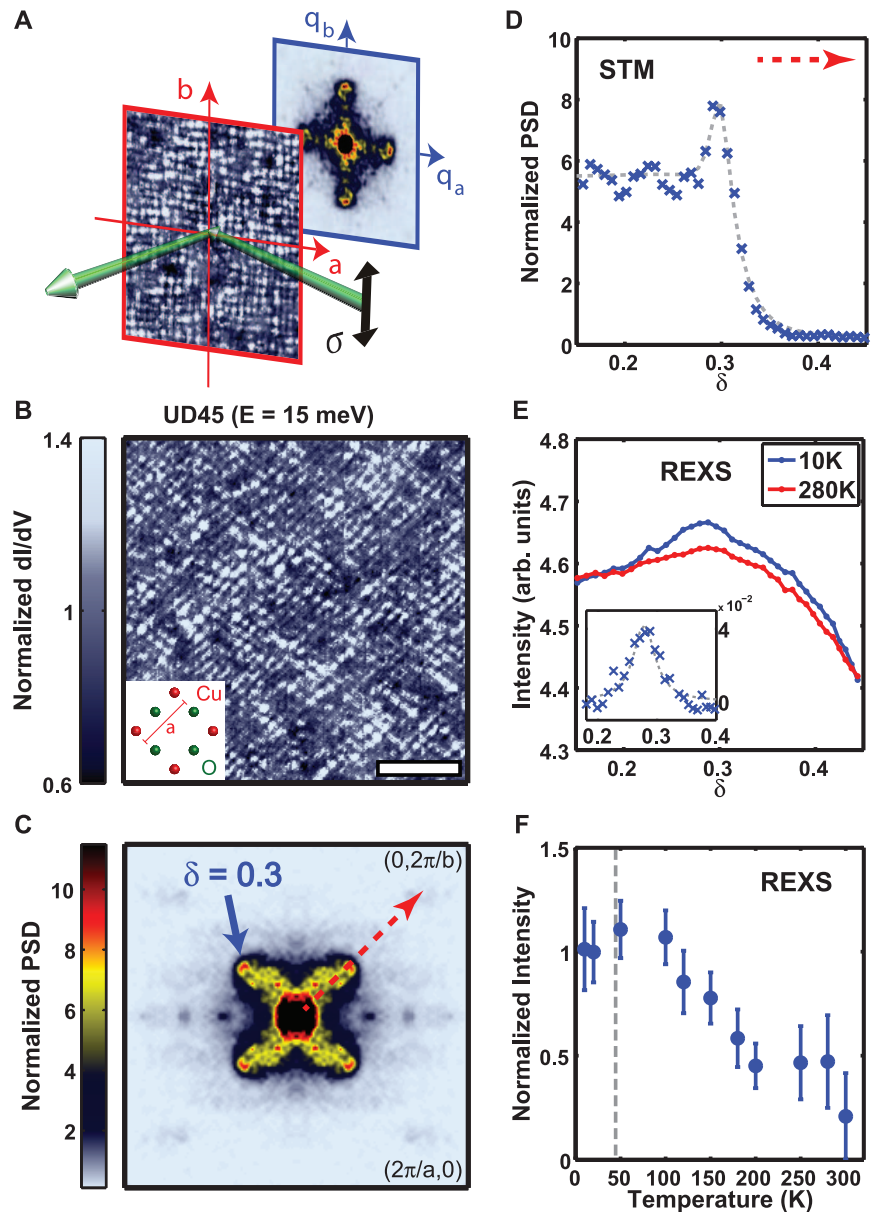


Fig. 1. CO in Bi-2212. (A) Schematic of the combined experimental STM-REXS approach. REXS experiments were performed with vertically polarized photons (σ) in a horizontal scattering geometry (22) and yield momentum space information corresponding to the real space modulations seen by STM (front) or, more directly, to the discrete Fourier transform of the STM real space data (back). We use the tetragonal crystal structure, where $a = b \approx 3.8$ Å represents the nearest neighbor Cu-Cu distance. (B) STM conductance map (15 meV, normalized to its spatial average) on a UD45 sample measured at 30 K. (Inset) The direction of the Cu-O-Cu bond direction relative to the measurement. Scale bar, 140 Å. (C) Discrete Fourier transform of the conductance map in (B); the corners of the image represent the atomic Bragg peaks at $(\pm 2\pi/a, 0)$ and $(0, \pm 2\pi/b)$. The discrete Fourier transform is mirror-symmetrized and normalized to its average value. The color scale represents the power spectral density (PSD) normalized to the $\delta = 0.3$ peak (blue arrow), which corresponds to the real space modulations seen in (B). The central peak ($\delta < 0.2$) corresponds to long wavelength inhomogeneity of the conductance map. (D) Line-cut of the energy-integrated (0 to 50 meV) discrete Fourier transform along the Cu-O bond direction [(C), red arrow] showing a clear peak at $\delta = 0.30$. Dashed line represents fit to a Lorentzian plus a background (22). (E) REXS δ -scans (22) for positive δ at low (blue) and high (red) temperatures on the same sample as in (B), showing a clear enhancement near $\delta = 0.3$ at low temperatures. (Inset) Background subtracted 10 K data, where the dashed line represents a Lorentzian fit to the data (22), in the same units as (E). (F) The REXS intensity extracted from the background-subtracted peak maxima as a function of temperature (22). The vertical dashed line corresponds to $T_c = 45$ K.

energies (small δ) to points near the nodes at low energies (large δ) (13, 18, 24, 25). The Fermi surface of this sample, like other underdoped cuprates, is gapped at the antinodal region because of the formation of the pseudogap at high temperatures T^* (which is well above T_c for this sample). Contrasting STM data above and well below T_c shows, first, that the same quasiparticles involved in the CO at high temperature are associated with superconducting pairing at low temperatures and, second, that those quasiparticles occupy regions near the ends of the Fermi arcs (Fig. 3E) (22, 23). The fact that such a competition occurs away from the antinodes suggests that charge organization is sec-

ondary to the formation of the pseudogap phase (19).

The unusual appearance of CO only when tunneling electrons into the sample from the STM tip (only for energies above the chemical potential), and its absence in ARPES studies, suggests that the nature of this organization is related to the strong electronic correlations, which have long been predicted to give rise to asymmetric tunneling in the doped Mott insulators (26, 27). In a simplified picture, if holes doped in a Mott insulator organize into patterns, tunneling electrons into the sample at low energies would be much easier into the hole sites as opposed to electron sites because double occupancy is energetically

unfavorable. In contrast, tunneling electrons out of the sample can easily occur at any site because either electrons are already present or can hop there from a nearby site on the lattice. Whether such constraints on the tunneling processes in a Mott system can explain the broad resonance (+20 meV) that we observe above the Fermi energy (which is remarkably independent of doping) requires further investigations. Overall, although much remains to be understood about the underlying mechanism of CO or its unusual characteristics reported here, its universality across cuprate families establishes it as a critical component for understanding the electronic properties and superconductivity of high- T_c cuprates.

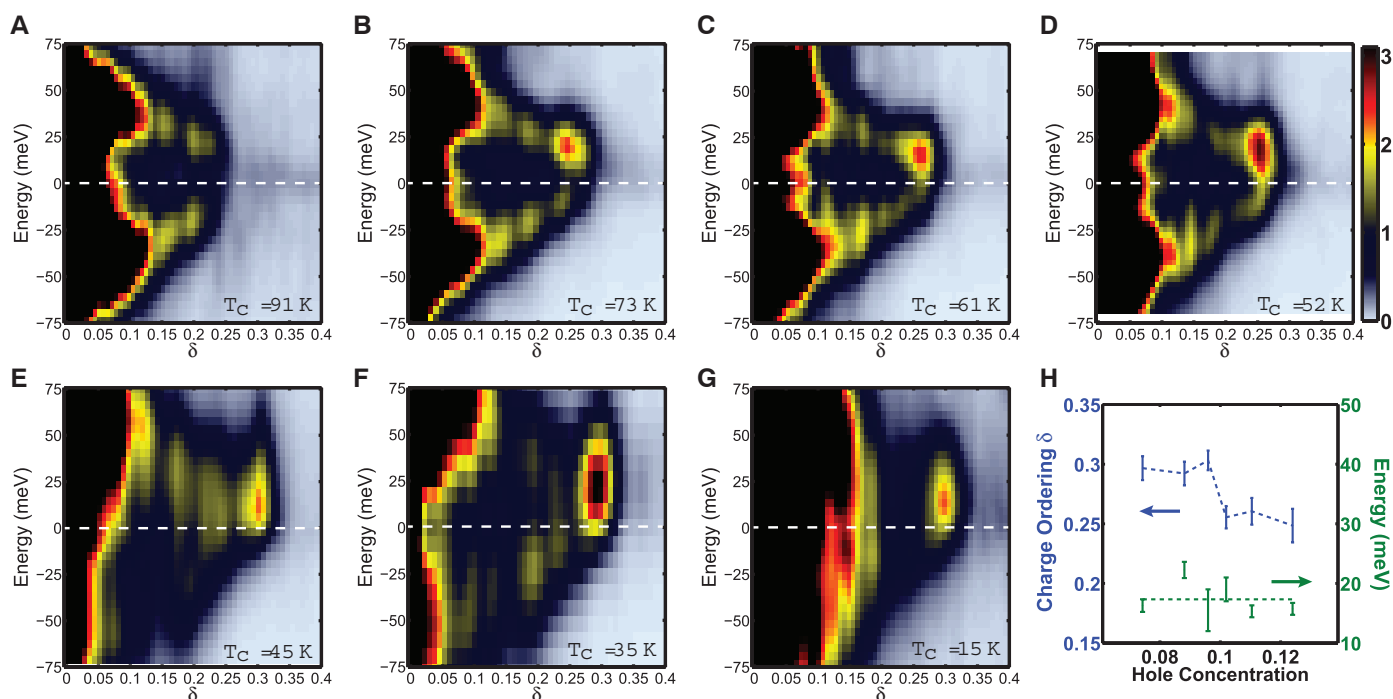


Fig. 2. Doping dependence of the STM data. (A to G) Energy-momentum structure of the modulations seen in STM along the Cu-O bond direction, extracted from line cuts along the $(2\pi/a, 0)$ direction of the discrete Fourier transforms measured at 30 K for different doping levels (22). (H) Energy and δ location of the CO feature as a function of

doping, extracted from energy-integrated δ cuts (22). The error bars in δ represent the half width at half maximum of the extracted peaks. The error bars in energy represent the SE obtained from a least-squares fit to a Lorentzian. The green dashed line represents the energy location averaged from all dopings.

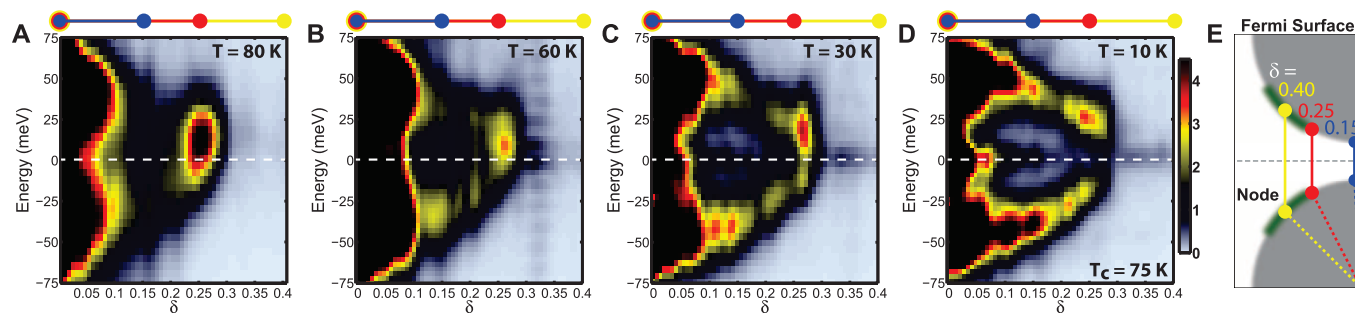


Fig. 3. Temperature dependence of the STM data. (A to D) Energy-momentum structure of the modulations seen in STM along the Cu-O bond direction, extracted from line cuts along the $(2\pi/a, 0)$ direction of the discrete Fourier transforms for a UD75 sample measured at selected temperatures. The data show the opposite temperature dependence between the particle-hole

symmetric BdG-QPI and the particle-hole asymmetric CO. (E) Schematic layout of the Fermi surface in Bi-2212 UD75 sample. The green segments represent the Fermi arc as determined by ARPES above T_c (22, 28). The vertical lines [also reproduced horizontally in (A) to (D)] correspond to QPI wave vectors connecting the Fermi surface [which is consistent with ARPES (22, 29)] at different regions.

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Acknowledgments: The work at Princeton was primarily supported by a grant from the U.S. Department of Energy (DOE) Basic Energy Sciences. The instrumentation and infrastructure at the Princeton Nanoscale Microscopy Laboratory used for this work were also supported by grants from NSF-DMR1104612, the NSF–Materials Research and

Engineering Center program through Princeton Center for Complex Materials (DMR-0819860), the Eric & Wendy Schmidt Transformative Fund, and the W. M. Keck Foundation. Work at BNL was supported by DOE under contract DE-AC02-98CH10886. The Max Planck–UBC Centre for Quantum Materials and Canadian Institute for Advanced Research Quantum Materials also supported this work. We thank P. W. Anderson, E. Abrahams, S. Kivelson, S. Misra, and N. P. Ong for fruitful discussions. We also acknowledge A. Damascelli and B. Keimer for discussions and for sharing the results of their x-ray studies on Bi-2201 before publication. A.Y. acknowledges the hospitality of the Aspen Center for Physics, supported under NSF grant PHYS-1066293. The synthesis of the oxygen-doped Bi2212 samples was supported by the Center for Emergent Superconductivity, an Energy Frontier Research Center.

Supplementary Materials

www.sciencemag.org/content/343/6169/393/suppl/DC1
Materials and Methods

Figs. S1 to S9

References (30, 31)

19 July 2013; accepted 25 November 2013

Published online 19 December 2013;

10.1126/science.1243479

Imaging Dynamics on the F + H₂O → HF + OH Potential Energy Surfaces from Wells to Barriers

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Hua Guo,^{3†} Robert E. Continetti^{1†}

The study of gas-phase reaction dynamics has advanced to a point where four-atom reactions are the proving ground for detailed comparisons between experiment and theory. Here, a combined experimental and theoretical study of the dissociation dynamics of the tetra-atomic FH₂O system is presented, providing snapshots of the F + H₂O → HF + OH reaction. Photoelectron-photofragment coincidence measurements of the dissociative photodetachment (DPD) of the F[−](H₂O) anion revealed various dissociation pathways along different electronic states. A distinct photoelectron spectrum of stable FH–OH complexes was also measured and attributed to long-lived Feshbach resonances. Comparison to full-dimensional quantum calculations confirms the sensitivity of the DPD measurements to the subtle dynamics on the low-lying FH₂O potential energy surfaces over a wide range of nuclear configurations and energies.

Thanks to the fruitful interplay between experiment and theory, our understanding of elementary atom-diatom reactions such as F + H₂ → HF + H at the quantum mechanical level is now highly sophisticated (1, 2). As the number of atoms *N* in a chemical reaction increases, the complexity grows dramatically as 3*N* − 6 coordinates are necessary to fully describe the system. With six degrees of freedom, four-

atom reactions have emerged naturally as the new proving ground for understanding reaction dynamics. Recently, theoretical methods have progressed to a level where highly accurate comparisons with experimental results are becoming possible for four-atom reactions (3), such as the benchmark H₂ + OH → H₂O + H and its isotopologs (4–7).

One of the key foundations for analyzing a chemical reaction is a detailed knowledge of the Born-Oppenheimer potential energy surface (PES), which represents the relation between a geometrical arrangement of the nuclei of a chemical system and its corresponding electronic energy. A number of experimental techniques have been developed that are sensitive to different aspects of the molecular PES. For example, rate coefficients depend on the height of reaction barriers and/or

are sensitive to the long-range forces between reactants. Molecular spectroscopy provides structural information and allows probing of different product internal states. Crossed-molecular beam measurements of angle-resolved differential cross sections map the reaction dynamics on a quantum state-to-state level (8) and are sensitive to dynamical (e.g., Feshbach) resonances in chemical reactions (9). In addition, nonadiabatic dynamics that involve transitions between multiple electronic PESs have also attracted increasing attention (10, 11). Negative ion photoelectron spectroscopy has been demonstrated as an effective tool (12) with application to both nonadiabatic effects (13) and resonance phenomena (14). The combined information from these different sources offers a stringent test for state-of-the-art theoretical methods, advancing our understanding of chemical reactions in more complex systems. Substantial progress in the field of reaction dynamics has thereby always been closely tied to the detailed study of benchmark systems like F + H₂. A natural extension to consider as we move to four-atom systems is the F + H₂O reaction.

The F + H₂O reaction is a prototypical hydrogen abstraction reaction between a radical and water. It represents a fundamental reaction of anthropogenic fluorine in Earth's atmosphere (15) and may also play a key role in the formation of HF that has recently been discovered in the interstellar media (16). The interaction of the F atom with water gives rise to three different PESs, two of which correlate with the F(²P_{3/2}) ground state (Fig. 1). In the adiabatic limit, the lower-lying ground (X) state corresponds to HF + OH(²Π_{3/2}), whereas the higher-lying excited (A) state leads to spin-orbit excited HF + OH(²Π_{1/2}) products (17). A reaction barrier separates van der Waals (vdW) complexes on the entrance and exit channel sides of the reaction (18, 19). Recent theoretical studies have shown that the prereaction

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complex, $\text{F}-\text{H}_2\text{O}$, may have a strong influence on the reactivity of the system (20).

An early study of the $\text{F} + \text{H}_2\text{O}$ rate coefficient found no temperature dependence over a wide

range, implying a tunneling-mediated reaction at low temperatures (21). Wang and co-workers have probed the transition state of the $\text{F} + \text{H}_2\text{O}$ reaction using anion photoelectron spectroscopy

at 6.42-eV photon energy (22), and the fragmentation process has been analyzed by direct dynamics simulations (23). In an extensive set of crossed beam experiments, Nesbitt and co-workers have explored the $\text{F} + \text{H}_2\text{O}/\text{D}_2\text{O}$ reaction in great detail (17, 24–26), using optical methods to probe the internal state distributions of the nascent diatomic products. Although the collision energies in these experiments were too low to overcome the adiabatic barrier for the formation of spin-excited OH, a branching ratio of ${}^2\Pi_{3/2}:{}^2\Pi_{1/2} = 0.69:0.31$ was observed, suggesting the importance of nonadiabatic dynamics beyond the Born-Oppenheimer picture in this system (25).

Here, we report on a joint experimental and theoretical study of the FH_2O system. Unlike crossed beam experiments, where reaction products are only probed asymptotically, the photoelectron-photofragment coincidence experiment used here provides snapshots of the reaction dynamics all along the reaction coordinate, from the saddle point accessed by photodetachment to the asymptotic product channels as determined by translational spectroscopy. As shown in Fig. 1, the wave function for the $\text{F}^-(\text{H}_2\text{O})$ anion has substantial overlap with configurations ranging from the saddle point to the vdW wells on the neutral reaction surface. This makes a comparison with state-of-the-art quantum dynamics calculations possible for a broad range of observables, illustrating the great strides being made in understanding the PES and dynamics of four-atom systems.

In the experiment, a pulsed laser at 258 nm (4.80 eV) was used to photodetach an electron from $\text{F}^-(\text{H}_2\text{O})$ in a fast ion beam, and the photoelectron was collected in a velocity map imaging spectrometer. The photofragments resulting from this process were collected in coincidence on a

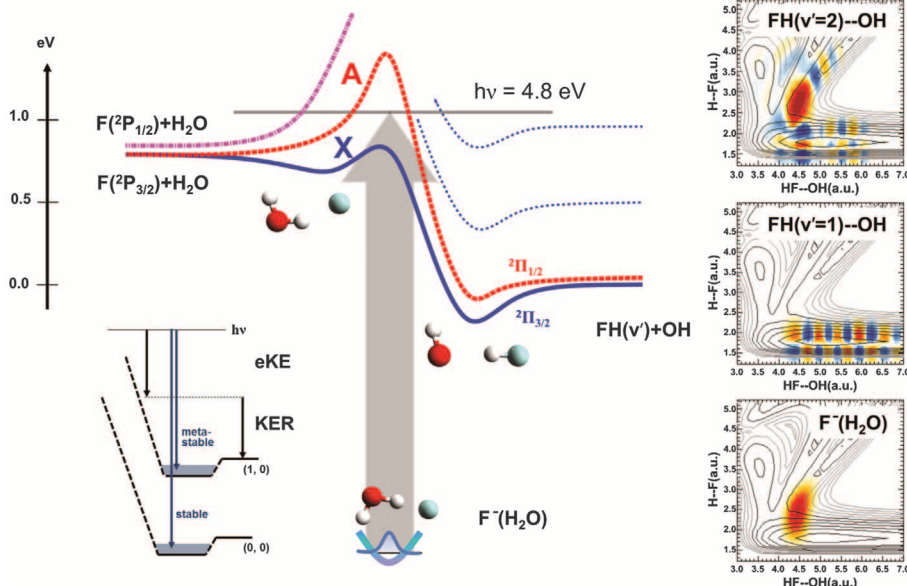


Fig. 1. Potential energy surfaces of the ground (X) and excited (A) states of the FH_2O system. In the PPC experiment, the $\text{F}^-(\text{H}_2\text{O})$ anion (as indicated schematically) is projected onto the neutral surfaces by laser photodetachment. The energy of the ejected photoelectron (eKE) is measured in coincidence with the kinetic energy release of the photofragments (KER). A photoelectron spectrum of nondissociative events is measured by enforcing coincidence between electrons and stable FH_2O products only. The panels on the right show wave functions for the $\text{F}^-(\text{H}_2\text{O})$ anion and Feshbach resonances for $\text{FH}(v' = 1)\text{--OH}$ and $\text{FH}(v' = 2)\text{--OH}$ states on the X state, with coordinates described in the SM. The energetic contours on the X state surface are spaced by 0.2-eV contours, with the darker contours representing energetically allowed regions at the photon energy used. The wave functions in the two Jacobi coordinates are obtained with other coordinates fixed at the maximal probability.

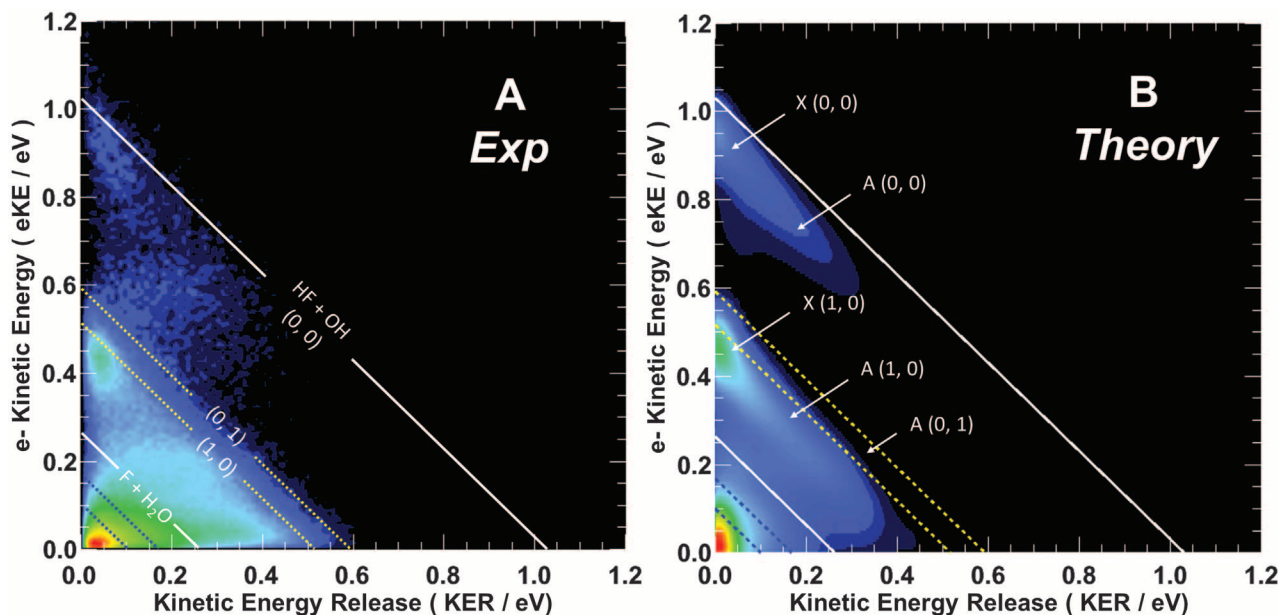


Fig. 2. Photoelectron-photofragment coincidence spectra for the FH_2O system. (A) Measured and (B) calculated spectra are compared. The solid white lines mark the energetic limits for dissociation into $\text{HF} + \text{OH}$ (upper line)

and $\text{F} + \text{H}_2\text{O}$ (lower line) fragments. The dashed lines indicate vibrationally excited product states. White arrows in (B) show regions of notable contributions for specific channels.

time- and position-sensitive detector to determine the translational energy release from the dissociation. The photoelectron-photofragment-coincidence (PPC) technique allows the “half reaction” (i.e., the fragmentation into either reactants or products) to be measured in a kinematically complete fashion (27, 28). The PPC spectrum was obtained by recording the electron kinetic energy (eKE) and photofragment kinetic energy release (KER) for each event in a two-dimensional (2D) coincidence plot (Fig. 2A). The overall energy available in the dissociative photodetachment (DPD) process can be distributed into either electron kinetic energy or photofragment energy (kinetic and internal). The total kinetic energy (hereafter simply called “total energy”) determines the internal energy of the products, and therefore their final states. All combinations of eKE and KER that lead to the same final states give rise to a diagonal band structure that emerges from the spectrum. The maximum total energy available in the dissociation is given by the photon energy, the energy necessary to photodetach an electron from $F^-(H_2O)$, and the energy released in the fragmentation (29). $HF + OH$ products reach this energetic cutoff when all available energy is converted into translational energy. This energetic cutoff is marked by the outermost diagonal white line in Fig. 2.

The PPC spectrum in Fig. 2 reveals many details of the dissociation dynamics. Although the experimental resolution is not sufficient to distinguish different rotational levels in the products, high rotational excitation can be inferred from the width of the observed vibrational bands. At total energies of 0.59 and 0.52 eV, formation of $HF(v' = 0) + OH(v' = 1)$ and $HF(v' = 1) + OH(v' = 0)$ products, denoted (0, 1) and (1, 0), respectively, becomes possible. These energetic limits are marked by the yellow dotted lines in the spectrum that assume single vibrational quanta in the OH and HF products. Both product states are observed as soon as they become energetically possible. Once these product channels open up along the eKE coordinate, the (0, 0) products die off, indicating that dissociative events starting from configurations close to the barrier for the reaction (at low eKE) favor production of vibrationally excited states. This HF vibrational excitation is a consequence of substantial lengthening of the H-F distance in the transition state relative to the equilibrium bond length of the free HF product.

The energetics for populating (0, 2), (2, 0), and (1, 1) final states are marked by the diagonal lines located at 0.18, 0.11, and 0.09 eV total energy, respectively. At a total energy of 0.25 eV, fragmentation into $F + H_2O$ becomes accessible, as marked by the lower white line in the image. Under the current experimental conditions, the $F + H_2O$ product channel cannot be separated from the formation of $HF + OH$ owing to limited product mass resolution. Analyzing the vibrational state distributions, we find that only 8% of all products are observed in the (0, 0) ground state. A majority of all events (52%) are found in

the energetic range between the opening of the (0, 1) and the (0, 2) product channels. This result is consistent with recent crossed beam experiments for the $F + H_2O$ reaction where a population inversion for the $HF(v = 1)$ stretch mode was observed (26).

All events in the (0, 0) band are observed in two distinct regions of the PPC spectrum. One region is centered around high eKE (0.9 eV) and low KER (0.05 eV), the other around lower eKE (0.6 eV) and higher KER (0.2 eV). Both regions are found in a diagonal band structure representing the same total energy and therefore lead to the same final states. The pattern is mirrored in the (1, 0) band with a higher (0.45 eV) and a lower (0.15 eV) eKE region. This finding points to two different channels for the dissociation process and, as discussed below, reflects different electronic states that participate in the dissociation.

To test our ability to predict and understand the experimental results, we have modeled the dissociation process using full-dimensional quantum dynamics calculations, based on ab initio PESs of the two lowest-lying electronic states of FH_2O (Fig. 1) (18, 19). Mimicking the experiment, the anion wave function in its ro-vibrational ground state on an ab initio-based anion PES is vertically projected on the neutral PESs and propagated with a full-dimensional Hamiltonian ($J = 0$). Both the $F + H_2O$ and $HF + OH$ asymptotic states are resolved after propagation of ~ 2 ps. The final-state distributions at the photon energy are then used to construct the 2D PPC spectrum (see supplementary materials, SM). Overall, the agreement between the experimental data and theoretical results is very good (Fig. 2B). The topology of the spectrum, as well as the relative population of

the various fragmentation channels, is well reproduced, indicating that the theory captures the essence of the dynamics of FH_2O dissociation. The calculations allow decomposition of the PPC spectrum into its final-state resolved components (figs. S2 to S4), which sheds further light on the features observed in the experiment. Indeed, the theoretical results confirm the large preponderance of (1, 0) products (66% of all products at this photon energy), in agreement with the experimental results. The outgoing wave function becomes temporarily trapped in a number of Feshbach resonance states (Fig. 1). This resonance-mediated dissociation is directly correlated with vibrationally excited HF products. In addition, the theoretical results confirm that the A state plays a substantial role in the dissociation dynamics of FH_2O and is responsible for the weak features, with low eKE and high KER in each channel. By contrast, the fraction for the $F + H_2O$ channel is found to be small (12%) and exclusively originates from the X state.

Complementary to the dissociative channels, long-lived complexes in the FH_2O system were identified by enforcing coincidence of the outgoing photoelectron with single particles arriving at the neutral detector with the mass of the parent anion, yielding a photoelectron spectrum of stable neutral complexes (Fig. 3). This kind of information cannot be obtained in a photoelectron spectroscopy measurement, where the fate of the neutral products is not recorded. The existence of such a spectrum in the FH_2O system is per se quite remarkable as any metastable states must have a lifetime of ≥ 5 μ s to be detectable in the experiment. The observed stable spectrum in Fig. 3 features a double peak structure at 1.0 and 1.15 eV.

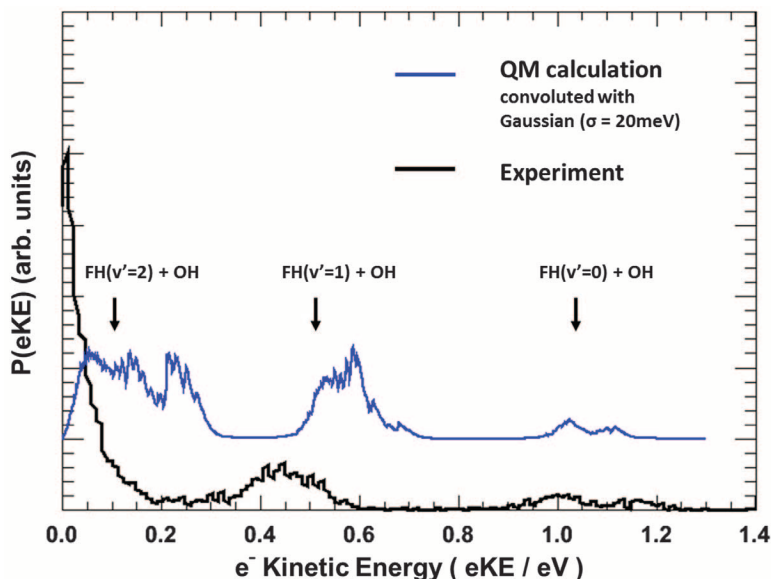


Fig. 3. Experimental and theoretical stable photoelectron spectra for the FH_2O system, showing the normalized electron probability distribution as a function of eKE. The peaks in both spectra at ~ 1.15 eV are assigned to a direct transition into bound states in the stable $FH-OH$ vdW well. The peaks at ~ 0.5 eV in both spectra are assigned to Feshbach resonance states trapped in the $FH(v' = 1)-OH$ vdW well. QM, quantum mechanical.

The peak associated with the highest eKE in the spectrum is found beyond the energetic limit for fragmentation into OH + HF ground state products. These events must result from direct photodetachment into the exit channel FH–OH vdW complex of the F + H₂O reaction. A higher-lying HF–HO vdW complex in the exit channel may be responsible for the lower-energy peak at 1.0 eV (19). Another feature is observed at 0.45 eV, roughly one vibrational quantum in HF (~0.6 eV) above the FH–OH vdW complex. Corresponding features observed in the dissociative PPC spectrum in Fig. 2 suggest that this signal arises from a temporarily trapped vdW complex, FH(v' = 1)–OH, a long-lived Feshbach resonance.

To understand the nature of the stable spectrum, the energy spectrum of the remaining wave packet after 2.5-ps propagation was computed. The calculated spectrum is compared in Fig. 3 with the experiment. The features centered around 0.55 and 0.15 eV are attributed to long-lived Feshbach resonances trapped in the FH(v' = 1,2)–OH vdW wells, with representative wave functions illustrated in Fig. 1. Given that the experimental spectrum represents states with far longer lifetimes (5 μ s) than is tractable to theoretically compute, the agreement is quite reasonable. An intriguing finding is the peak at 0.05 eV in the calculated stable spectrum. A closer inspection of the co-incidence plot in Fig. 2A reveals a corresponding horizontal feature at the same eKE, corresponding to events distributed between KER = 0 to 0.4 eV. The appearance of this feature in conjunction with the peak observed in the stable spectrum can be interpreted in terms of a metastable state that is formed in the DPD process [e.g., an FH(v' = 2)–OH Feshbach resonance] and therefore has a fixed electron spectrum. However, as this state decays on its way to the detector, the delayed fragmentation process over a nanosecond-microsecond time scale leads to an underestimation

of the KER of the fragments, resulting in a horizontal band structure.

The wide range of observables simultaneously captured in the PPC experiment reported here provides a critical test for the accurate description of both the PESs and reaction dynamics over a wide range of translational and internal energies. Both lowest-lying electronic states play an active role in the dissociation process and must be taken into account to understand the nature of the FH₂O dissociation. Long-lived Feshbach resonances have been found in this four-atom system, experimentally and theoretically. Although very good overall agreement has been achieved between experiment and theory, challenges remain. The discrepancies found in the contribution of the A state, as well as the energetic positions and nature of the Feshbach resonance states, demand further studies of this new benchmark reaction.

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Acknowledgments: This work was supported by the U.S. Department of Energy (DE-FG03-98ER14879 to R.E.C. and DE-FG02-05ER15694 to H.G.). R.O. thanks the German Academic Exchange Service (DAAD) for a postdoctoral research fellowship. J.M. thanks the National Natural Science Foundation of China (21303110) for partial support.

Supplementary Materials

www.sciencemag.org/content/343/6169/396/suppl/DC1
Materials and Methods
Figs. S1 to S5
Tables S1 to S4
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21 October 2013; accepted 13 December 2013
Published online 9 January 2014;
10.1126/science.1247424

Strong Ground Motion Prediction Using Virtual Earthquakes

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Sedimentary basins increase the damaging effects of earthquakes by trapping and amplifying seismic waves. Simulations of seismic wave propagation in sedimentary basins capture this effect; however, there exists no method to validate these results for earthquakes that have not yet occurred. We present a new approach for ground motion prediction that uses the ambient seismic field. We apply our method to a suite of magnitude 7 scenario earthquakes on the southern San Andreas fault and compare our ground motion predictions with simulations. Both methods find strong amplification and coupling of source and structure effects, but they predict substantially different shaking patterns across the Los Angeles Basin. The virtual earthquake approach provides a new approach for predicting long-period strong ground motion.

Sedimentary basins amplify and extend the duration of strong shaking from earthquakes (1). State-of-the-art simulations of wave

propagation for scenario earthquakes through crustal structures that include sedimentary basins explore these effects (2–7). Evidence that strong

basin amplification in Los Angeles for earthquakes that would occur on the southern San Andreas fault emerged from such ground motion simulations (3, 7), but because of the absence of recent earthquakes large enough to excite long-period seismic waves, they have not been validated with observations. Our study was motivated by the need to validate these simulations, which, if correct, would greatly increase seismic hazard.

To validate ground motion simulations without recordings of large earthquakes, we developed

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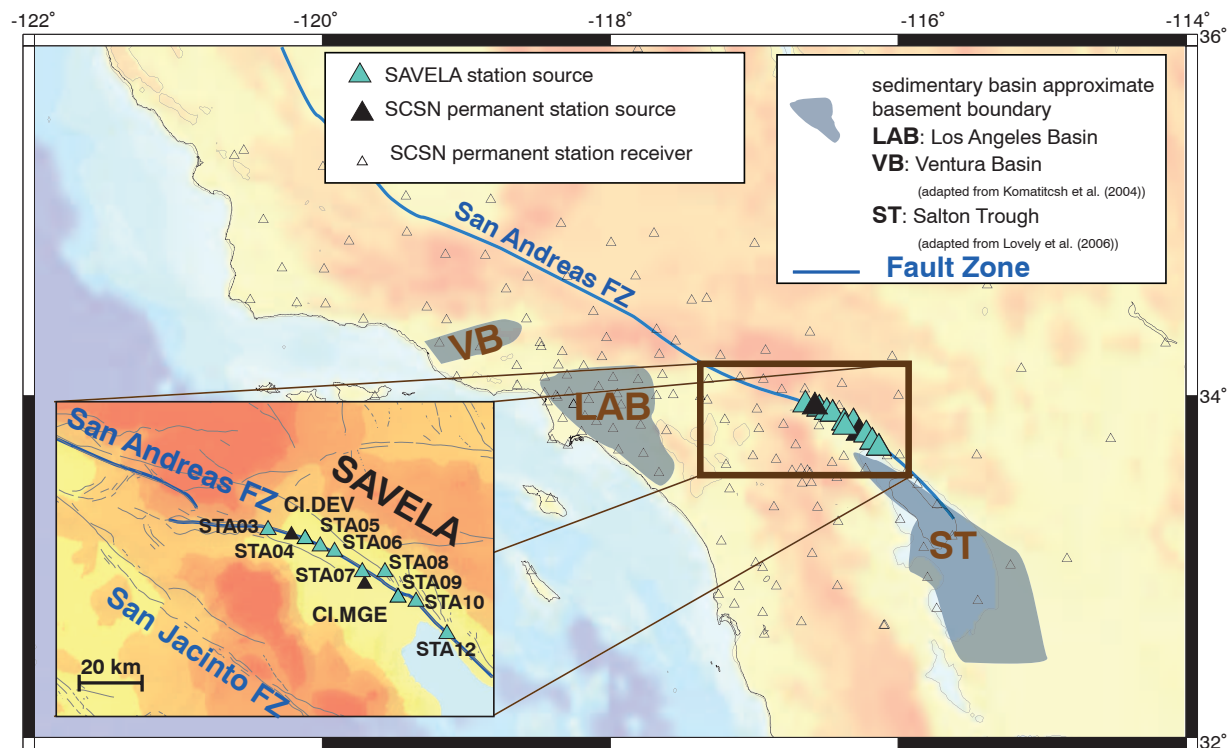


Fig. 1. Temporary SAVELA and permanent Southern California Seismic Network (SCSN) stations. Shaded area shows the approximate shape of major sedimentary basins. Open triangles are SCSN seismic stations that we treat as receivers. Filled triangles are seismic stations with temporary deploy-

ments (blue) and permanent SCSN (black) stations near the San Andreas fault that we treat as virtual sources. San Andreas fault zone (solid blue lines) and quaternary faults location from <http://earthquakes.usgs.gov/hazards/qfaults>. SAVELA stations are located within 3 km of the fault trace (bottom left inset).

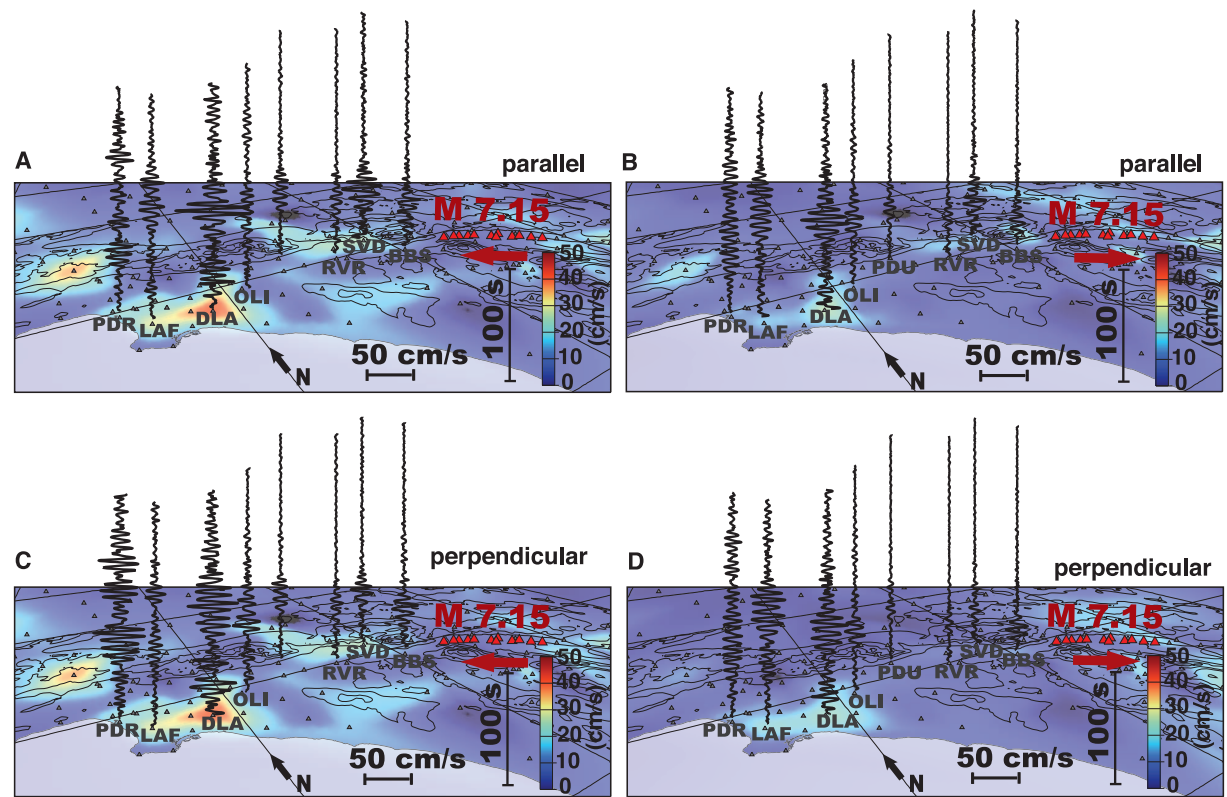


Fig. 2. Seismic amplification in sedimentary basin and waveguide effect. Velocity seismograms (black waveforms) and PGV (color scale) for two M 7.15 scenario earthquake ruptures determined using the VEA. Simulations are divided into northwestward-propagating ruptures (A and C) and southeastward-

propagating ruptures (B and D) for the horizontal fault-parallel (A and B) and fault-perpendicular (C and D) components of motion. Seismic amplification appears at all cases but is stronger for northwestward rupture that produces up to two times more shaking than the southeastward rupture.

a Virtual Earthquake Approach (VEA) that models long-period strong ground motion using Green's functions derived from the ambient seismic field (fig. S1). The ambient seismic field is excited by the coupling of the oceans and atmosphere with the solid Earth and carries the signature of the structure between two seismic stations. It is possible to extract the Earth's response to an impulsive force (i.e., the Green's function) through correlation of the ambient seismic field (8–15). This capability has enabled the imaging of the Earth's wave speed structure through travel-time measurements (16, 17). The ambient seismic field also contains amplitude information, which has been used to measure anelastic effects (18, 19) and which we exploit to predict earthquake ground motion intensity.

Ambient seismic field Green's functions reproduce waveform shapes and relative amplitudes of earthquakes for nearby stations (20). Once the Green's functions are corrected from a surface point-force source to a double-couple source at depth, the agreement between real-earthquake

and virtual-earthquake waveforms systematically improves (21). Here, we extended this method from point sources, appropriate for moderate magnitude ($M \sim 5$) earthquakes, to finite ruptures of larger magnitude using the representation theorem (22).

We applied this technique to predict ground motion in greater Los Angeles for scenario earthquakes on the San Andreas fault. To support this effort, we deployed a temporary array of broadband seismometers along the fault to determine the Green's functions for virtual sources along that segment (Fig. 1). We refer to our experiment as SAVELA (San Andreas Virtual Earthquake—Los Angeles). We corrected the ambient-field Green's functions for source location, depth, and mechanism using our understanding of the surface-wave excitation local to the virtual source (23–25). We then calculated seismograms using the VEA for a suite of $M \sim 7$ scenario earthquakes along the fault and compared the results with simulations of wave propagation through three-dimensional models of the Earth's crust (6). We

explain the details of the method in the supplementary materials.

The source of the ambient seismic field is not uniformly distributed with azimuth (26), and different components of motion (S_H and $P-S_v$) are not equally excited. Both of these factors affect the recovered amplitude. We corrected our Green's functions to compensate for a first-order azimuthal pattern as follows. For each source component (vertical, radial, and transverse), we find sinusoidal functions that best match the observed variation with azimuth of the Green's function amplitudes (fig. S2). For each component, we estimated the calibration factor required for the ambient-field data to predict observed amplitudes for two local moderate events—the 2008 M 5.4 Chino Hills and 2008 M 5.1 Hector Road earthquakes.

We considered an ensemble of 96 magnitude 7.15 simulated ruptures developed for this segment of the fault. This represents a small subset of pseudodynamic (27) models developed for the CyberShake project (6). The kinematic source model describes fault rupture as a collection of

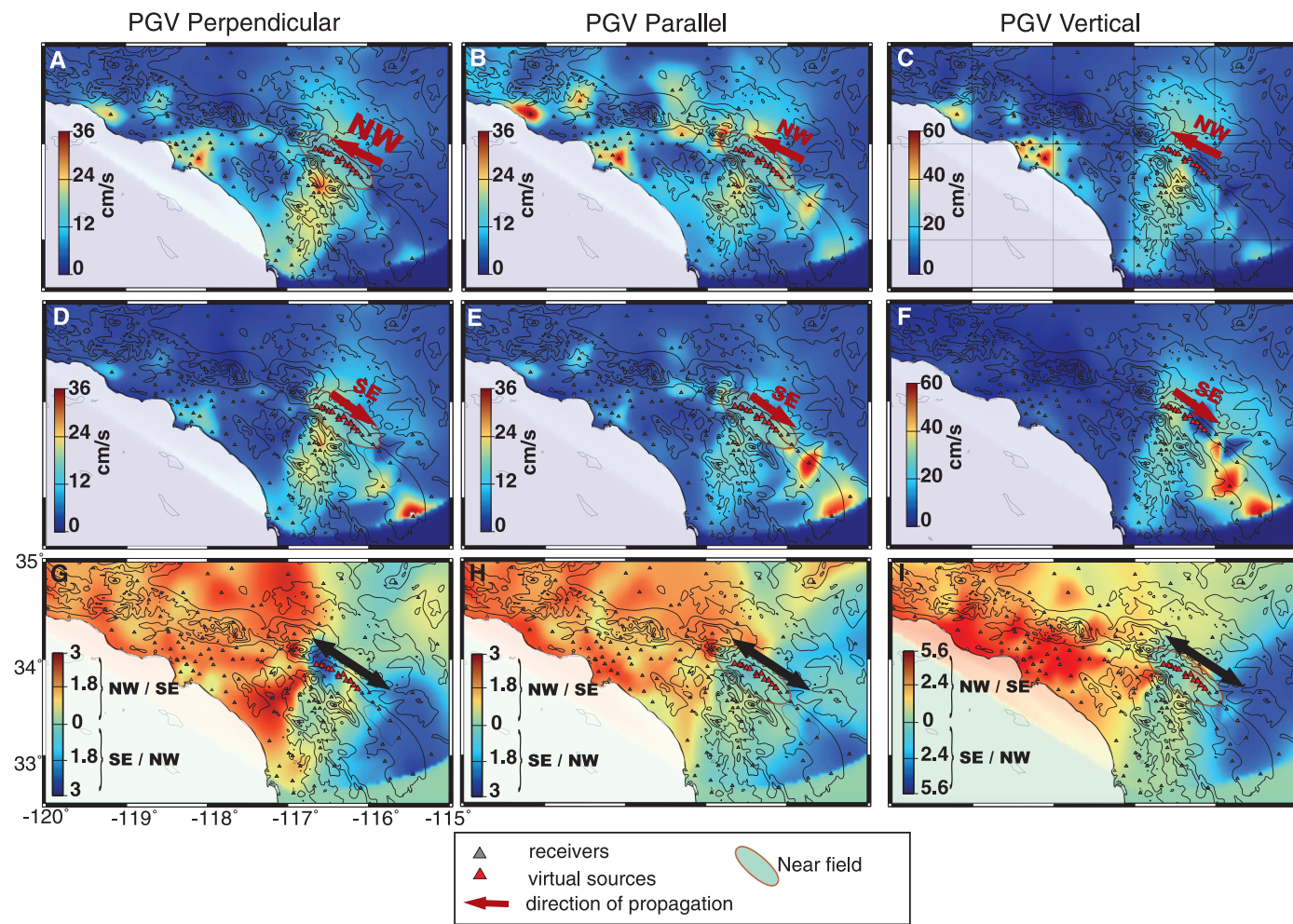


Fig. 3. Coupling of source-directivity and basin structure. PGV averaged over all northwestward-NW (A to C) and southeastward-NE (D to F) propagating ruptures for perpendicular-to-strike (A and D), parallel-to-strike (B and E), and vertical (C and F) components of motion. Los Angeles sedimentary basin

experiences seismic amplification in all cases, and the effect is stronger for the parallel components. (G to I) Ratio of averaged peak amplitudes of NW-over-SE propagating ruptures. (G to I) Warm colors show how many times the NW ruptures enhance the shaking as compared to the SE ruptures.

point sources that represents square fault patches of 1-km² area. We use the VEA correction to account for depth, mechanism, and timing of each. We convolve the waveform with the source time function of individual sources and sum the contributions from all point sources to form the entire source. We compare long-period ground motion predictions that result from our VEA with the CyberShake simulations.

Our ground motion predictions show strong seismic amplification in the Los Angeles sedimentary basin compared to surrounding areas (Fig. 2). We compare the effect of unilateral rupture for a given slip distribution, by assuming hypocenters located at the southern and northern ends of the segment. In both cases, we find seismic amplification in downtown Los Angeles with peak amplitudes up to three times larger than in surrounding areas. The pattern of peak ground motion and the persistence of the amplification in both predictions reflect the presence of a waveguide that funnels seismic waves from west of

San Geronio Pass to downtown Los Angeles. The amplification is more pronounced for the rupture propagating toward the basin, and in this example, the ratio of predicted peak velocities is approximately three. This contrast is less than predicted by the TeraShake simulations (3) and may result from the fact that the *M* 7.7 TeraShake source model ruptured a much greater fault length.

Ensembles of slip distributions that have hypocenters located at either end of the fault segment show a similar source-directivity effect on ground motion (Fig. 3). Amplification in sedimentary basins appears clearly in all cases. Our results again show peak amplitudes in Los Angeles that are, on average, three times those of the surroundings (Fig. 3). The long-period peak ground velocity (PGV) averaged over all scenarios, experienced in downtown Los Angeles, is as high as 50 cm/s for the vertical and 36 cm/s for the horizontal components. This differs substantially from the strong ground motion amplitudes at higher fre-

quencies and closer distances where horizontal amplitudes are typically larger than vertical amplitudes (28).

We construct Green's functions for a laterally homogeneous medium (25) and find a systematic variation of the amplitudes that approximates a dipole (supplementary text and fig. S3). We contrast this with the behavior for virtual-earthquake seismograms, from which we see strong variations of amplitude ratios for all three components of motion (Fig. 3). The strength of the directivity-structure coupling is not only larger than for the laterally homogeneous case but is more extensive and spatially variable. The coupling between source directivity and basin structure (6) appears in the enhanced amplification in both the Ventura and Los Angeles basins. Northern Los Angeles and the San Gabriel foothills experience strong coupling on the strike-perpendicular component, whereas the coupling of the parallel component is stronger in South Los Angeles, Palos Verdes, and Chino Hills. Coupling of the vertical component

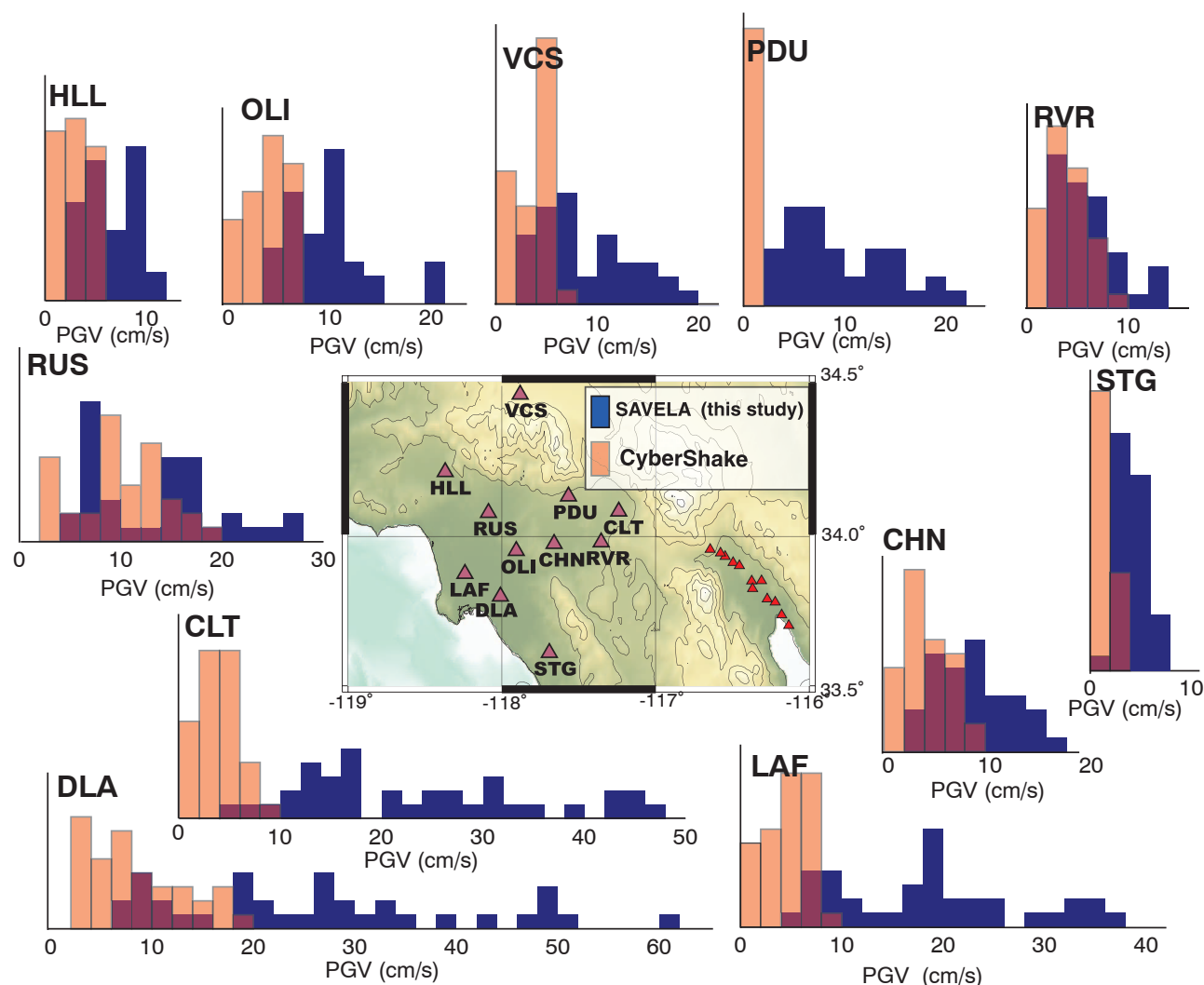


Fig. 4. Cross-validation of simulations (CyberShake) and the VEA (SAVELA). Histograms of PGV (*y* axis, number of scenario earthquakes) for all 36 tested scenarios for CyberShake (orange) and SAVELA (blue) for particular sites in Los

Angeles Basin. SAVELA predicts equal shaking for RUS, RVR, and HLL and predicts larger shaking in particular for sites in the basin (LAF, DLA, CHN, CLT, and STG). The variability of the ground motion increases with intensity of shaking.

correlates with the deepest part of the sedimentary basin.

The predicted ground motion is qualitatively similar for the VEA and CyberShake simulations (figs. S4 and S5); however, there are substantial quantitative differences in the level and distribution of the shaking (Fig. 4). SVELA results show stronger shaking than CyberShake at most sites. This is especially true for receivers in the basin. We note that both methods are subject to substantial uncertainties. The accuracy of the CyberShake simulations depends strongly on the accuracy of the assumed crustal velocity model and its derived Green's functions. The accuracy of the virtual earthquake results depends strongly both on the accuracy of the ambient-field Green's functions and on the accuracy of the amplitude calibration. The Green's functions we used for this study (fig. S1) would be more accurate if we had data from a longer deployment. To estimate the variability in PGV due to the amplitude calibration described above, we measure the maximum difference in predicted PGV when using the calibration from either the Hector Road or Chino Hills earthquake to the mean of the calibration used in this study. The variability that results is bounded at 10 cm/s for our simulations. For both the virtual earthquake and CyberShake simulations, the variation of the PGV is narrow for stations with low PGV values (bedrock sites) and wide for stations with high PGV values (basin sites). The coefficient of variation is approximately constant for SVELA (fig. S7), which indicates that variability increases proportionally with ground motion amplitude.

Nonlinear effects in shallow materials are important in strong ground motion. Ground motion simulations that have incorporated nonlinear soil effects (29) have found a large decrease in the predicted strong ground motion. This could be an important effect for the scenarios we consider because unconsolidated sediments are likely to be found in sedimentary basins and would be expected to behave nonlinearly during strong shaking. We calibrated the amplitudes of the Green's functions, such that the peak amplitudes predicted by our approach matched those of moderate-sized earthquakes. Our approach, as well as the CyberShake simulations, is based on an assumption of linearity. To the extent that nonlinear effects are important, our predicted ground motion amplitudes are likely to overestimate true amplitudes in future large earthquakes.

We confirm the presence and the influence of a waveguide to the west of San Geronio Pass that funnels seismic waves from San Andreas fault events into the Los Angeles Basin. This amplification is present for all tested scenarios. We also confirm that directivity couples with shallow crustal structure to increase basin amplification (6). We find a constant coefficient of variation, which means that shaking variability is proportional to shaking intensity. We also find a wider range of predicted peak amplitudes than is found in simulations, which would increase

uncertainty in ground motion predictions and thereby impact seismic hazard assessments. We note, however, that there are substantial uncertainties in our estimated Green's functions and their amplitude calibration. Moreover, station coverage in the basin is sparse, and we have only sampled a small portion of the variability expected for a complex wavefield in the basin. Our results support more ambitious, targeted experiments to improve the accuracy of long-period strong ground motion prediction for future earthquakes in regions subject to high seismic risk.

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Acknowledgments: We thank K. Olsen and anonymous reviewers for their comments and contributions to improve the manuscript. This work was supported by NSF grant EAR-0943885 and by the Southern California Earthquake Center (SCEC). SCEC is funded by NSF cooperative agreement EAR-0529922 and U.S. Geological Survey cooperative agreement 07HQAG0008. The SCEC contribution number for this paper is 1812. Data are available in the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/343/6169/399/suppl/DC1
Materials and Methods
Figs. S1 to S7
References (30–33)

9 September 2013; accepted 19 December 2013
10.1126/science.1245678

Increased Dust Deposition in the Pacific Southern Ocean During Glacial Periods

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Dust deposition in the Southern Ocean constitutes a critical modulator of past global climate variability, but how it has varied temporally and geographically is underdetermined. Here, we present data sets of glacial-interglacial dust-supply cycles from the largest Southern Ocean sector, the polar South Pacific, indicating three times higher dust deposition during glacial periods than during interglacials for the past million years. Although the most likely dust source for the South Pacific is Australia and New Zealand, the glacial-interglacial pattern and timing of lithogenic sediment deposition is similar to dust records from Antarctica and the South Atlantic dominated by Patagonian sources. These similarities imply large-scale common climate forcings, such as latitudinal shifts of the southern westerlies and regionally enhanced glaciogenic dust mobilization in New Zealand and Patagonia.

Mineral aerosols (dust) play a crucial role in determining the pattern and magnitude of climate variability. Dust im-

purities trapped in Antarctic ice point to ~25 times higher glacial dust fluxes compared with interglacials (1). It has been suggested that an increase

in the atmospheric supply of iron (Fe) by dust during glacial periods may have stimulated marine productivity in the Southern Ocean (SO), contributing to the reduction of atmospheric CO₂ concentrations observed during ice ages (2). Dust-induced Fe fertilization represents one key mechanism that potentially affects past ocean-atmosphere CO₂ exchange (3–5), although the magnitude with respect to other SO processes such as sea-ice extent, overturning strength, and water column stratification is still under debate (5, 6). Antarctic ice cores allow only an indirect qualitative inference of dust deposition over the ocean and cannot be used to quantitatively estimate dust deposition in the different SO sectors. Marine sediments, however, provide a direct estimate of SO dust deposition and marine export production. To date, marine studies are primarily confined to the Atlantic SO sector located downstream of Patagonia, a strong dust source during glacial periods. In that region, substantially enhanced glacial dust fluxes and subantarctic productivity have been interpreted to control, at most, one-third to one-half of the observed glacial-interglacial atmospheric CO₂ difference (4, 7), consistent with similar results based on the phasing of dust and CO₂ fluctuations in the Epica Dome C (EDC) ice core (8). However, this finding is based on the assumption that dust deposition and Fe fertilization take place equally in the entire Subantarctic Zone. Biogeochemical models with geographically variable dust fields for the SO suggest a somewhat lower CO₂ reduction (9).

Glacial dust recorded in ice cores from the East Antarctic Plateau originates almost exclusively from South America, including Patagonia (10). Model simulations (11) have supported this view and have suggested that dust deposition in the SO occurs mainly in the Atlantic and western Indian SO sectors. However, a recent simulation indicates that stronger glacial Australian dust sources resulted in a distinct glacial dust deposition field covering most of the Pacific SO sector but only reaching marginal areas of the Antarctic continent (12) (fig. S1). To date, sediment data on modern and glacial dust deposition in the Pacific SO are restricted to proximal sites around Australia, especially in the Tasman Sea (13) and subordinately east of New Zealand (14). These records show a substantial increase in the deposition of terrigenous material during the Last Glacial Maximum (LGM) compared with the present Holocene, but proximal

marine dust records are not necessarily representative of the pelagic Pacific SO. From the open Pacific Ocean, a number of short sediment records reveal enhanced lithogenic fluxes during the LGM (15, 16). However, the contribution of dust remains inconclusive, because most of the coring sites are located in waters potentially affected by the deposition of ice-rafted detritus (IRD) (17).

Here, we report dust flux reconstructions over the past million years based on a suite of sediment cores across the western and central Pacific SO, recently collected during the German research vessel (R/V) *Polarstern* expedition ANT-XXVI/2 (18) (Fig. 1 and fig. S2). On the basis of benthic oxygen isotope stratigraphy and biostratigraphic age control points (19) (fig. S3), Fe-content changes in our sediment records reveal clear similarities to dust content variations documented in Antarctic ice cores and Atlantic SO sediments (fig. S4). We therefore aligned our high-resolution Fe content records to the EDC dust content record (1) back to ~800 thousand years before the present (ky B.P.) and to the Fe content record of Ocean Drilling Program (ODP) Site 1090 (7) for the interval extending beyond the reach of Antarctic ice cores (19). The similar pattern in the Fe and dust contents on glacial-interglacial time scales over the past million years in our Pacific SO sediment records (Fig. 2) extends from the Permanently Open Ocean and Polar Frontal Zone in

the southern southwest Pacific Basin eastwards to the central Subantarctic Pacific (fig. S2). This geographic pattern largely follows the modeled modern Australian dust plume beneath the maximum westerly winds (20, 21) (Fig. 1) and also simulations of the LGM deposition pattern (12) (fig. S1) and is consistent with a primarily eolian origin of Fe supply to the Pacific SO. Though we cannot exclude minor input of IRD, the strong similarity between Fe content changes and the supply of terrestrial n-alkanes in two cores (fig. S5) strongly supports the predominant eolian origin. Moreover, IRD would not be distributed homogeneously over large distances and thus would not create the consistent variations, as shown by our sediment records across the western and central Pacific SO (Fig. 2).

We selected two sediment cores from the suite of ANT-XXVI/2 cores to quantify mass accumulation rates of the lithogenic fraction (MAR_{Litho}) and paleoproductivity estimates [biogenic barium (Ba_{ex}) and opal (19)]. Core PS75/76-2 covers the past ~1000 ky and was recovered close to the Subantarctic Front in the southwest Pacific. Core PS75/59-2 reaches back to ~480 ky B.P. and is located in the Subantarctic Zone of the central South Pacific (Fig. 1 and fig. S2). MAR_{Litho} were obtained by calibrating the high-resolution Fe content records with lithogenic contents calculated from the concentration of ²³²Th. As with the Fe content records, the shape and glacial-interglacial pattern of the Pacific SO MAR_{Litho} records reveal

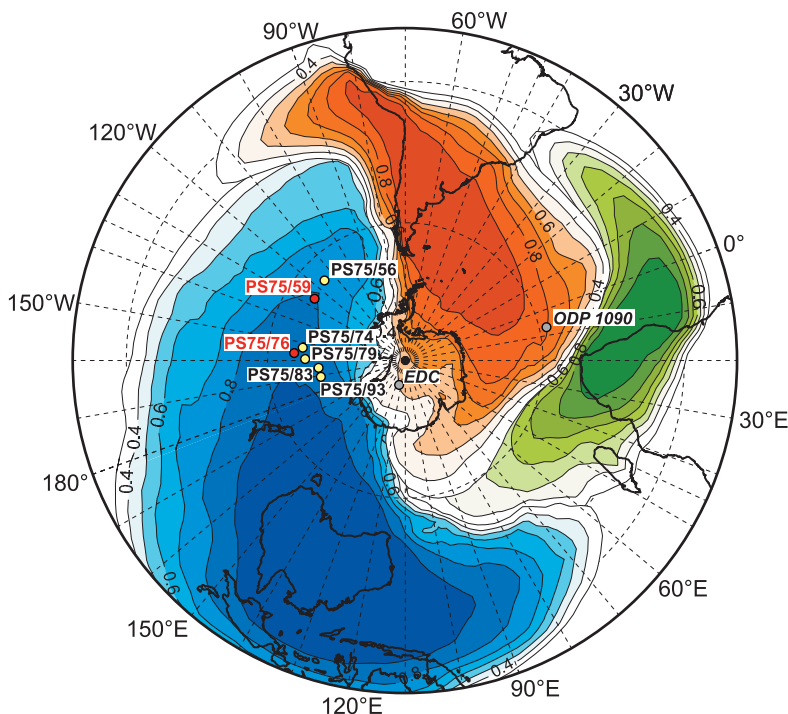


Fig. 1. Map showing the modern relative contributions of the three major dust sources in the Southern Hemisphere (blue, Australia; red, South America; green, South Africa), based on model data (20). Red dots mark primary core locations; yellow dots indicate additional cores; gray dots denote location of published reference records (1, 4, 7).

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strong similarities to the EDC ice core dust MAR and Atlantic SO MAR_{Litho} records from ODP Site 1090 (Fig. 3). Glacial MAR_{Litho} are substantially higher and exceed interglacial values by a factor of ~3 to 15 (fig. S6).

However, fluxes of both biogenic and lithogenic material in the SO may be substantially affected by sediment focusing below the vigorous Antarctic Circumpolar Current (22). To correct vertical fluxes for the potential influence of lateral sediment redistribution (focusing), we applied the ^{230}Th normalization method [limited to the past ~300 ky B.P. (19)]. Glacial $^{230}\text{Th}_{\text{norm}}$ MAR_{Litho} tend to be considerably lower than

the non-normalized values (Fig. 3, B and C). For each site, the ^{230}Th -normalized MAR_{Litho} show internally consistent variability across the past three glacial-interglacial cycles, indicating a ~threefold increase in lithogenic fluxes during glacials (Fig. 3E and fig. S6), comparable to glacial increases at low latitudes (23). The absolute $^{230}\text{Th}_{\text{norm}}$ MAR_{Litho} values decrease from west (156°W) to east (125°W) (table S1). The downwind decrease of $^{230}\text{Th}_{\text{norm}}$ MAR_{Litho}, recorded in our cores, is consistent with a primarily eolian source of the lithogenic material, originating from Australia, as also shown in dust simulations (12, 20). However, our interglacial

$^{230}\text{Th}_{\text{norm}}$ MAR_{Litho} are somewhat higher than lithogenic particle fluxes from sediment traps and water samples in the southwest Pacific SO (24, 25) and model-based estimates for our core sites (12, 20). In contrast, higher Holocene Australian dust fluxes have been derived from New Zealand peat bogs (26) (table S1).

Our $^{230}\text{Th}_{\text{norm}}$ MAR_{Litho} and $^{230}\text{Th}_{\text{norm}}$ n-alkane MAR records (Fig. 3, E and F) document a ~threefold increase of dust supply to the Pacific SO during glacials (Fig. 3E and fig. S6), consistent with two previous $^{230}\text{Th}_{\text{norm}}$ MAR_{Litho} records from the central Subantarctic Pacific SO (16) (Fig. 3E) and a compilation of other Pacific dust deposition records (27). Taken together, these sediment data suggest a considerable enhancement of glacial dust input to the Pacific SO. Though absolute Pacific glacial dust fluxes are ~50% lower than in the Atlantic SO, the relative glacial-interglacial increase is almost as large. Considering the large geographic extension of the Pacific SO, this threefold increase in Pacific SO dust fluxes requires a substantial enhancement of the Australian dust source, considerable changes in atmospheric circulation, or an additional dust source not active during interglacials.

Modeling of the contribution of the different Southern Hemisphere dust sources to modern dust deposition in the SO shows that more than 80% of modern dust deposition in the western and central Pacific SO originates from Australia (12, 20) (Fig. 1). The large-scale distribution of Southern Hemisphere dust is primarily achieved through transport within the westerly wind belt. The uptake of dust and transport and distribution through the westerly wind belt into the SO primarily occurs in austral winter and spring (21), when the westerlies extend further north. In contrast to dust originating from Patagonia, Australian dust plumes are transported in the free troposphere and can thus be distributed over very large distances (20). Although there is presently no consensus about glacial changes of the westerlies in climate models (28), most paleodata-based reconstructions imply a strengthening and/or equatorward shift of the wind belt (27). Together with more expanded arid dust source areas in Australia (29) and increases in gustiness in the source regions (30), a northward extent and/or enhancement of the westerlies over southeast Australia during glacials would plausibly increase the dust uptake and export into the Pacific SO. Further, it is conceivable that New Zealand acted as an additional dust source. The westerlies, enhanced by strong katabatic winds in the lee of the glaciated New Zealand Alps (31), could have transported glacial outwash material out into the Pacific SO, a similar mechanism to that proposed for Patagonian dust sources (32). Distinguishing between an enhanced Australian dust source and the emergence of New Zealand as a glaciogenic dust source will require additional geochemical information about the provenance

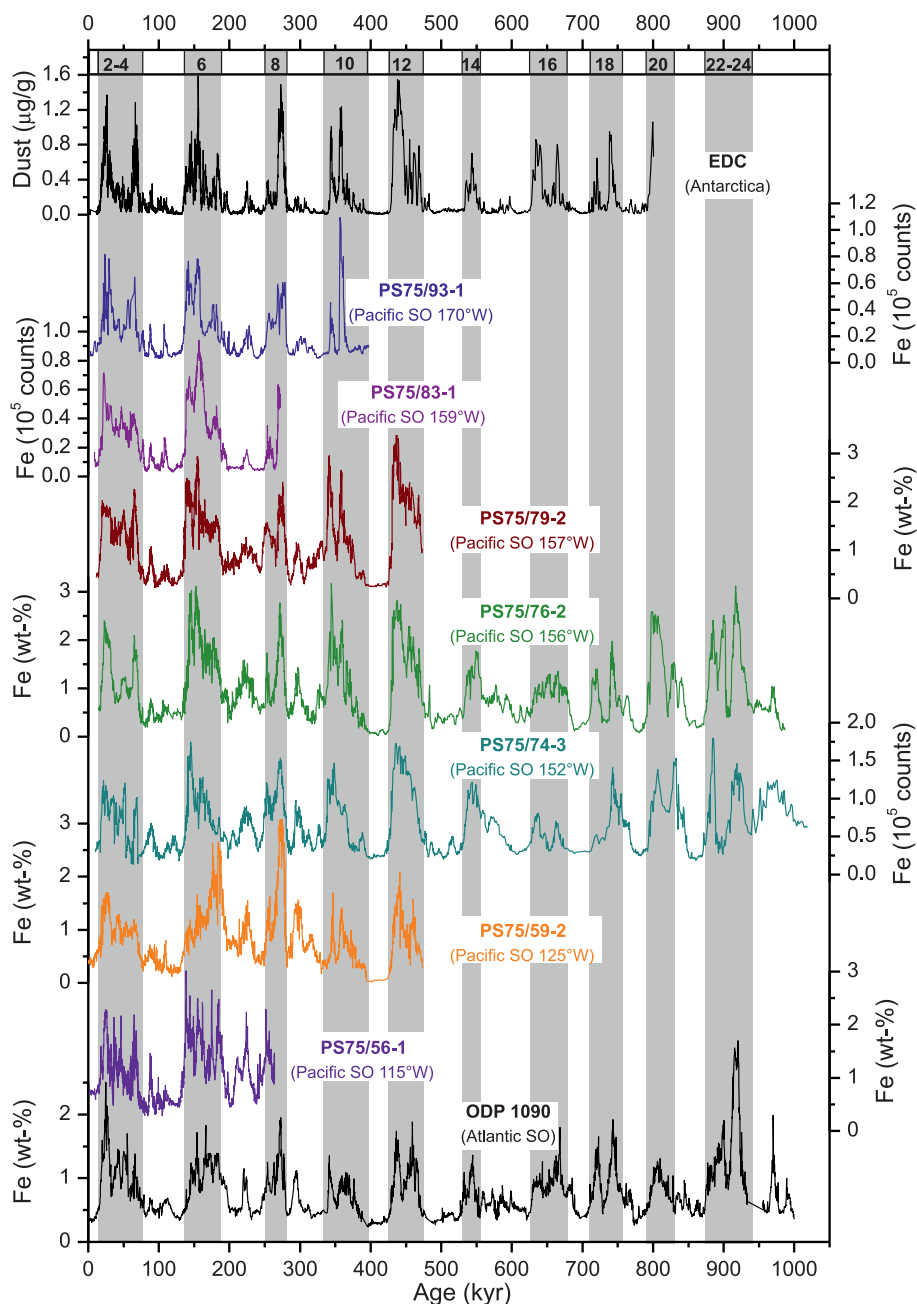


Fig. 2. Iron content fluctuations across the Pacific and Atlantic SO (7) compared to dust content changes in the EDC ice core (1).

of the dust exported to the SO Pacific during glacial times.

The ^{230}Th normalization method documents substantial sediment focusing at our two sites (fig. S7). Despite this effect of lateral sediment redistribution, the glacial-interglacial pattern and shape of dust fluctuations in the EDC ice core record are preserved in great detail [both in the MAR and Fe-content records (Figs. 2 and 3)]. This finding suggests that the climate-controlled variations in the availability of eolian material are the dominant factor in controlling the shape and pattern of glacial-interglacial lithogenic sediment accumulation, even at sites where focusing is substantial. A prominent feature of our PS75/76-2 $\text{MAR}_{\text{Litho}}$ beyond the range of $^{230}\text{Th}_{\text{norm}}$ is a decrease in glacial $\text{MAR}_{\text{Litho}}$ before marine isotope stage (MIS) 12 (~480 ky B.P.) (Fig. 3B and fig. S6). A very similar decrease is likewise seen in the Fe content record from core PS75/74-3, excluding a local origin of this feature (Fig. 2). This change coincides with a less pronounced Mid-Brunhes shift in glacial-interglacial amplitudes in the EDC and ODP Site 1090 records (Fig. 3, A and D, and fig. S6). Assuming that the shift in our $\text{MAR}_{\text{Litho}}$ is not entirely controlled by a systematic change in sediment focusing through bottom currents during the Mid-Brunhes interval, our data document reduced glacial dust input for the pre-MIS 11 interval. One explanation could be a different sensitivity of the Australian dust sources to glacial severity. In contrast to Patagonia, which lies at higher latitudes, Australian dust sources may require colder global temperature to achieve a similar increase to their Patagonian counterparts. Pre-MIS12 glacials were generally shorter than younger ones (1). Therefore, the time of high dust productivity may have been much shorter for Australian and New Zealand dust sources compared to Patagonian sources, explaining the more substantial decrease in pre-MIS 12 glacial Pacific dust fluxes.

Taken together, our sediment records document a substantial glacial dust supply from Australian and/or New Zealand sources to the Pacific SO sector eastward to at least 125°W . The ~threefold increase in glacial dust accumulation in the Pacific SO confirms the enhanced LGM-Holocene dust flux ratios from simulations (12) (fig. S1). Our Pacific SO data revise the current picture of Patagonia representing the almost exclusive source region for glacial dust deposited in the glacial SO. The previously unmeasured dust input to the largest SO sector during several glacial periods may have substantial implications for biological productivity on a global scale. Preliminary paleoproductivity estimates based on Ba_{exc} and biogenic opal records from our subantarctic core PS75/59-2 suggest ~two to three times higher glacial values; that is, the same order of magnitude as the dust increase (Fig. 4). This finding is consistent with other available sediment records from the Pacific SO (15, 16) that reveal generally higher opal MAR north of the modern Antarctic Polar Front during glacials. However, these data

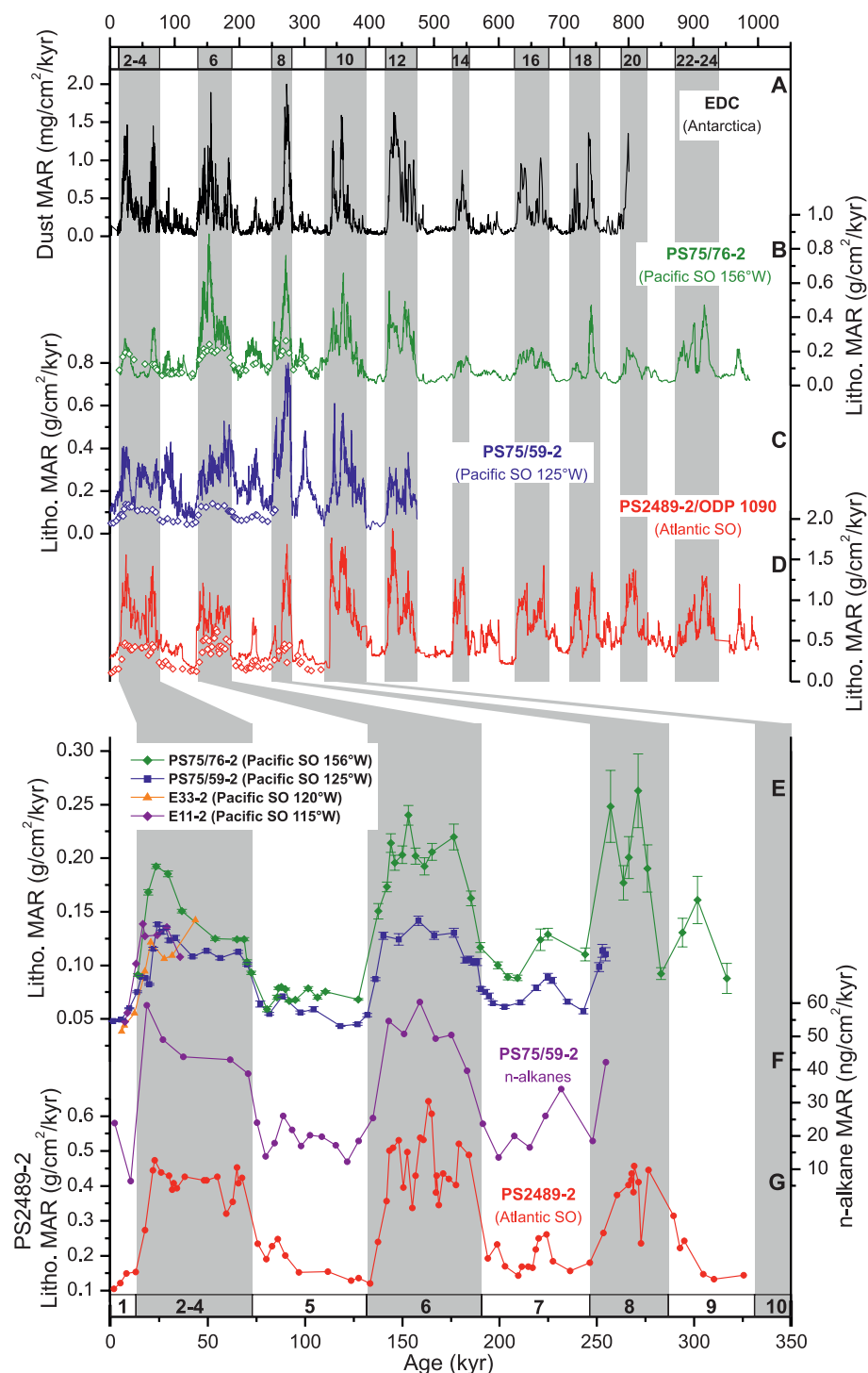


Fig. 3. Changes in lithogenic mass accumulation rates ($\text{MAR}_{\text{Litho}}$). (A) Dust MAR in the EDC ice core (1). (B) $\text{MAR}_{\text{Litho}}$ core PS75/76-2. (C) $\text{MAR}_{\text{Litho}}$ core PS75/59-2. (D) $\text{MAR}_{\text{Litho}}$ ODP Site 1090 (4, 7) [recalculated (19)]. Open diamonds in (A) to (C) show ^{230}Th -normalized $\text{MAR}_{\text{Litho}}$. (E) ^{230}Th -normalized $\text{MAR}_{\text{Litho}}$ values from cores PS75/59-2, PS75/76-2, E11-2, and E33-2 (16). (F) ^{230}Th -normalized C_{29} and C_{31} n-alkane MAR from core PS75/59-2. (G) ^{230}Th -normalized $\text{MAR}_{\text{Litho}}$ core PS2489-2 (4, 7). Vertical bars in (E) denote 1σ error.

also indicate that this increase is compensated by decreasing productivity southward, a pattern that has been suggested to extend across multiple glacial-interglacial cycles in the Atlantic SO (33).

Furthermore, opal MAR do not necessarily reflect productivity of the surface ocean and enhanced organic carbon export, particularly in the seasonal ice zone (3).

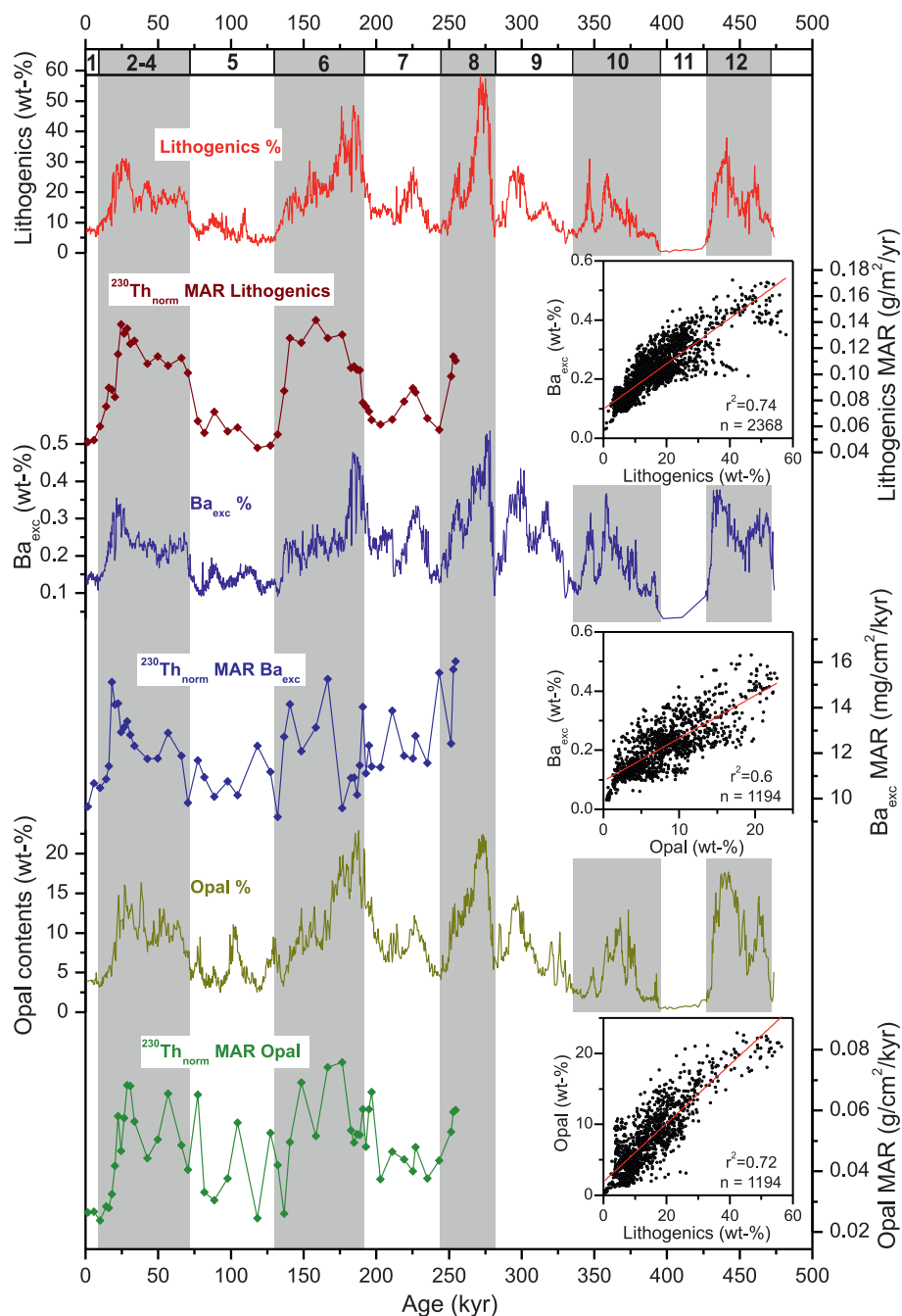


Fig. 4. Proxy records for paleoproductivity from subantarctic core PS75/59-2. Together with their contents, the ^{230}Th -normalized MAR records of Ba_{exc} , biogenic opal, and lithogenics are shown. Scatter diagrams (insets) show positive correlation between lithogenics, Ba_{exc} , and biogenic opal, suggesting that the paleoproductivity and lithogenic supply records largely covary. r^2 , correlation coefficient; n, number of samples.

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Acknowledgments: We thank the captain, crew, and scientific party of R/V *Polarstern* for their support during cruise ANT-XXVI-2. J. Collins, R. Tiedemann, H. W. Arz, and three anonymous reviewers provided comments and suggestions that substantially improved the paper. We acknowledge data contributions by P. Köhler and S. Steph and thank S. Albani and N. Mahowald for making their model output available. U. Bock, J. Heffer, R. Fröhling, and S. Wiebe provided technical support at AWI. G.W. thanks R. Schwarz and M. Q. Fleisher for excellent laboratory support at Lamont-Doherty Earth Observatory. We acknowledge financial support for this work through the AWI Helmholtz-Zentrum für Polar- und Meeresforschung, the MARUM—Center for Marine Environmental Sciences, the University of Bremen, and the European Union project Past4future. F. Lambert acknowledges support by Project CONICYT/FONDAP/15110009. Data are available at www.pangaea.de.

Supplementary Materials

www.sciencemag.org/content/343/6169/403/suppl/DC1
Materials and Methods
Figs. S1 to S10
Table S1
References (34–58)

2 September 2013; accepted 19 December 2013
10.1126/science.1245424

A Peptide Hormone and Its Receptor Protein Kinase Regulate Plant Cell Expansion

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Plant cells are immobile; thus, plant growth and development depend on cell expansion rather than cell migration. The molecular mechanism by which the plasma membrane initiates changes in the cell expansion rate remains elusive. We found that a secreted peptide, RALF (rapid alkalization factor), suppresses cell elongation of the primary root by activating the cell surface receptor FERONIA in *Arabidopsis thaliana*. A direct peptide-receptor interaction is supported by specific binding of RALF to FERONIA and reduced binding and insensitivity to RALF-induced growth inhibition in *feronia* mutants. Phosphoproteome measurements demonstrate that the RALF-FERONIA interaction causes phosphorylation of plasma membrane H⁺-adenosine triphosphatase 2 at Ser⁸⁹⁹, mediating the inhibition of proton transport. The results reveal a molecular mechanism for RALF-induced extracellular alkalization and a signaling pathway that regulates cell expansion.

Cell expansion is a fundamental cellular process driving plant growth, and regulation of its rate and direction is a highly dynamic process that changes during development, as well as during adaptation to variations in the external environment. To maintain appropriate cell expansion rates, plant cells fine-tune signal transduction pathways involving protein phosphorylation cascades catalyzed by protein kinases and protein phosphatases. For example, light and the growth-stimulating hormone auxin increase cell expansion rates and decrease apoplastic pH via phosphorylation of the penultimate Thr⁹⁴⁷ residue (1, 2) in a C-terminal regulatory domain of plasma membrane H⁺-adenosine triphosphatase (H⁺-ATPase).

RALF (rapid alkalization factor), a 5-kD secreted peptide first discovered with assays searching for endogenous plant peptides with the ability to increase the pH of the media of cultured cells (3, 4), also induces a rapid increase in cytoplasmic calcium (5). Among more than 30 RALF-like genes in the *Arabidopsis* genome (fig. S1), RALF is most highly expressed in roots and was suggested to be involved in root development (6, 7) (figs. S2 and S3). We report here on signaling events at the plasma membrane that involve a peptide-receptor interaction leading to the suppression of root cell elongation. RALF mobilizes calcium in a manner resembling the signal transduction pathways of animal peptide growth factors, wherein the factor binds to a cell surface receptor that then autophosphorylates (8). We demonstrate that FERONIA receptor-like kinase binds to RALF and initiates a downstream phosphorylation signaling cascade that inhibits plasma membrane H⁺-ATPase activity, increases apoplastic pH, and reduces cell elongation. We identify RALF and FERONIA as a ligand-receptor pair and provide a molecular mechanism for a FERONIA-

mediated growth-inhibitory signaling pathway that may also intersect with the innate immune response.

To obtain material for biochemical and genetic studies, we expressed RALF as an N-terminal 6×His fusion peptide in *Escherichia coli* that was purified to homogeneity using an affinity column and reversed-phase high-performance liquid chromatography (fig. S4, A to D). His-

tagged RALF was biologically active in cytoplasmic calcium mobilization assays (fig. S4C). As a negative control, we also produced and purified an inactive RALF analog, RALF(Δ2-8), which lacks seven amino acids at its N terminus and is a biologically inactive analog (figs. S4E and S5) (9). To examine RALF-induced rapid changes in phosphorylation of plasma membrane proteins, we performed mass spectrometry-based quantitative phosphoproteomic profiling using an ¹⁵N metabolic labeling technique (10, 11) (fig. S6A). Seedlings were treated with 1 μM RALF peptide or water as a control for 5 min, homogenized, and plasma membranes purified. For a biological duplicate, the samples were reciprocally labeled. Mass spectrometric analyses of phosphopeptides allowed the quantification of ~550 ¹⁴N/¹⁵N phosphopeptide pairs, with median linear ratio changes of 1.14 and 1.17 for samples A (¹⁴N RALF/¹⁵N control) and B (¹⁴N control/¹⁵N RALF), respectively (fig. S6, B and C; fig. S7, A and B). Among the phosphopeptides quantified, five proteins displayed a change in abundance, observed in a reciprocal manner, by a factor of at least 2.5 (table S1). Four of these proteins increased in abundance—FERONIA receptor kinase (pS871 and pS874, factor of 8.3 to 13.4, Fig. 1A); plasma membrane H⁺-ATPase 2 (AHA2; factor of 2.6 to 4.3, fig. S8); calcium-dependent protein kinase 9 (CPK9; factor of 4.0 to 12.1, fig. S9);

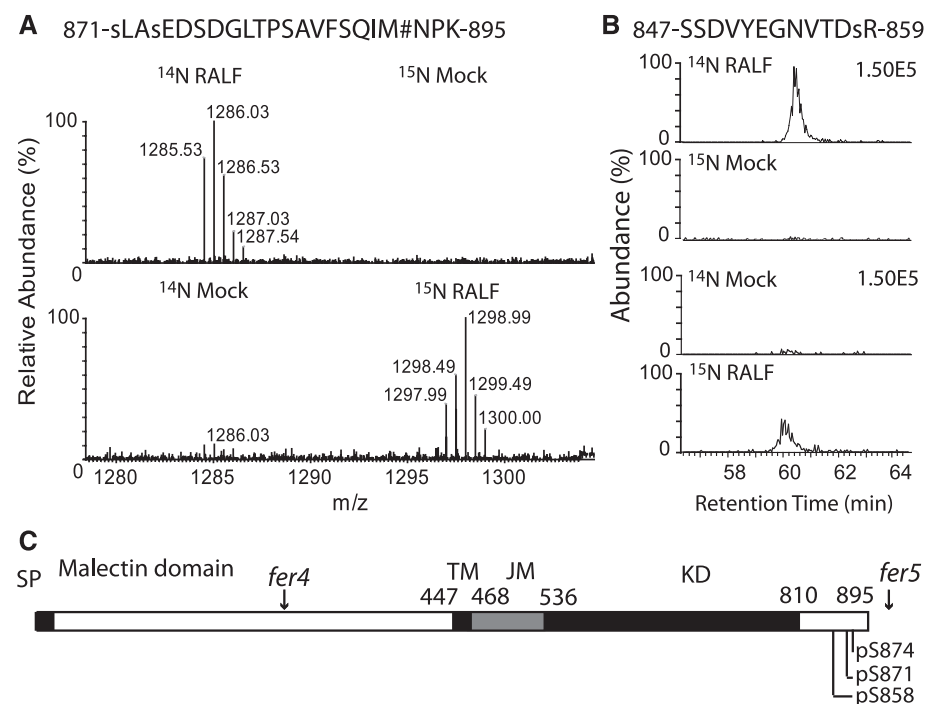


Fig. 1. RALF induces increased phosphorylation of FERONIA receptor kinase. (A) Mass spectrometric spectra showing an increased abundance of a FERONIA phosphopeptide containing pS871 and pS874 after RALF treatment. (B) Extracted ion chromatogram of a FERONIA phosphopeptide showing RALF-induced increase of phosphorylation at Ser⁸⁵⁸. (C) Structure of FERONIA receptor kinase. SP, signal peptide; TM, transmembrane domain; JM, juxtamembrane domain; KD, kinase domain. Arrows indicate the positions of T-DNA insertions in *fer* mutants. Abbreviations for amino acids: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; I, Ile; K, Lys; L, Leu; M#, oxidized Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; Y, Tyr; s, phosphorylated serine.

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and PEN3/ABCG36 transporter (factor of 4.17 to 4.52)—and one decreased: a second FERONIA-related receptor-like kinase we term ERULUS (ERU; factor of 0.22, fig. S10A).

We also observed the increased abundance of a FERONIA phosphopeptide containing a third serine phosphorylation site, pS858. Analysis was hampered by an unrelated peptide coeluting during the liquid chromatography–mass spectrometry analysis, but we manually reconstructed the chromatogram for the FERONIA phosphopeptide, SSDVYEGNVTDsR (Fig. 1B). The pS858 phosphopeptide increased by a factor of ~20 in the RALF-treated tissues. Using selective reaction monitoring with a heavy isotope-labeled synthetic phosphopeptide containing pS858 (fig. S7, C and D), we determined that pS858 is increased by a factor of 6.4 to 11.5 in RALF-treated seedlings. By analogy with the phospho-regulated mammalian epidermal growth factor receptor (12), we hypothesized that FERONIA was the receptor for RALF and that phosphorylation at the C terminus activated the kinase and initiated a RALF-induced signaling cascade (Fig. 1C).

The FERONIA receptor-like kinase was first described with *Arabidopsis* mutants defective in

pollen tube elongation arrest (13). During normal fertilization, the elongating pollen tube stops and releases its sperm nuclei at the egg, whereas in the *fer* mutant, the pollen tube overgrows and fails to release sperm nuclei, resulting in reduced fertility. FERONIA is among the most widely expressed members of the malectin receptor kinase family (14) (fig. S10B). In addition to changes in FERONIA, we detected another member of the malectin receptor family that responded to RALF, in this case with a decrease in abundance of the phosphopeptide containing pS497 located at the juxtamembrane domain (fig. S10C). Because FERONIA was named after the Etruscan fertility goddess, we have named this protein ERULUS, after a son of FERONIA in Etruscan mythology. To test the in planta function of these two receptor kinases (FERONIA and ERULUS) that show RALF-dependent changes in phosphorylation, we compared the growth of transferred DNA (T-DNA) knockdown (*fer5*) and knockout (*fer4*) mutants with that of the wild type in the presence and absence of RALF. At 1 μ M RALF, wild-type growth was inhibited, whereas the *fer4* null mutant was insensitive to RALF and the *fer5* knockdown mutant was moderately in-

hibited (Fig. 2A and fig. S11A). In contrast, T-DNA mutants of ERULUS showed shorter root hair phenotypes (fig. S10, D to G), but there were no differences in sensitivity to RALF relative to the wild type (Fig. 2B), despite changes in its phosphorylation in response to RALF. Inhibition of wild-type root elongation after RALF treatment was due to reduced cell elongation (fig. S11, B to D).

To characterize RALF-induced early cellular responses occurring within a few seconds after RALF treatment, we examined changes in cytoplasmic calcium of the *fer4* mutant using an aequorin-expressing *Arabidopsis* seedling as cytoplasmic calcium reporter. The RALF-induced calcium increase observed in the wild type was absent in the *fer4* mutant (Fig. 2C), although a control experiment with ATP treatment showed that the *fer4* mutant fully responds to this calcium-mobilizing agonist and is thus not generally defective in the calcium response machinery (fig. S12). These results indicate that the *fer4* mutant is specifically deficient in the RALF-dependent calcium signaling system.

RALF and FERONIA are both highly expressed in the mature zone of the root during the seedling stage (fig. S13), and their mRNA expression patterns and RALF-induced root growth arrest suggest that the two proteins act by restricting cell elongation in the post-elongation zone. Consistent with this idea, transcriptome analyses after RALF treatment for 30 min revealed that the families of genes encoding proteins known to be involved in cell expansion—SAUR63 [small auxin up RNA (15)], expansin (16), and the rate-limiting enzymes for biosynthesis of plant growth regulators, brassinosteroid-6-oxidase 2 (BR6OX2) and gibberellin-3-oxidase 1—were all down-regulated (fig. S14) (17, 18). Genes associated with calcium and ethylene signaling, including calmodulin and ethylene response factors (19), were up-regulated by RALF treatment. The RALF-induced change of BR6OX2 expression was absent in the *fer4* mutant (Fig. 2D).

A peptide derived from pathogenic bacterial flagellin, flg22, is sensed by the FLS2 receptor and also induces a phosphorylation change of AHA2 at Ser⁸⁹⁹ that coincides with apoplastic alkalization (20, 21). An AHA2 S899D (Ser⁸⁹⁹ → Asp) mutant expressed in yeast demonstrated that this phosphomimetic mutation reduced growth relative to wild-type AHA2; thus, an increase in AHA2 phosphorylation at Ser⁸⁹⁹ after RALF treatment is predicted to down-regulate H⁺-ATPase function, providing a molecular explanation for the observed RALF-induced apoplastic alkalization (22). As a check on specificity of the RALF-FERONIA interaction, we examined the flg22 sensitivity of the *fer* mutant as well as the RALF sensitivity of the *fls2* mutant. The *fer4* mutant was insensitive to RALF but not to flg22 peptide, and the *fls2* mutant was insensitive to flg22 but not to RALF (fig. S15, A and B). We selected 17 other lines containing knockout mutations for receptor-like kinases and cell surface receptor-like proteins that are implicated in RALF signaling because they are co-regulated with RALF or are

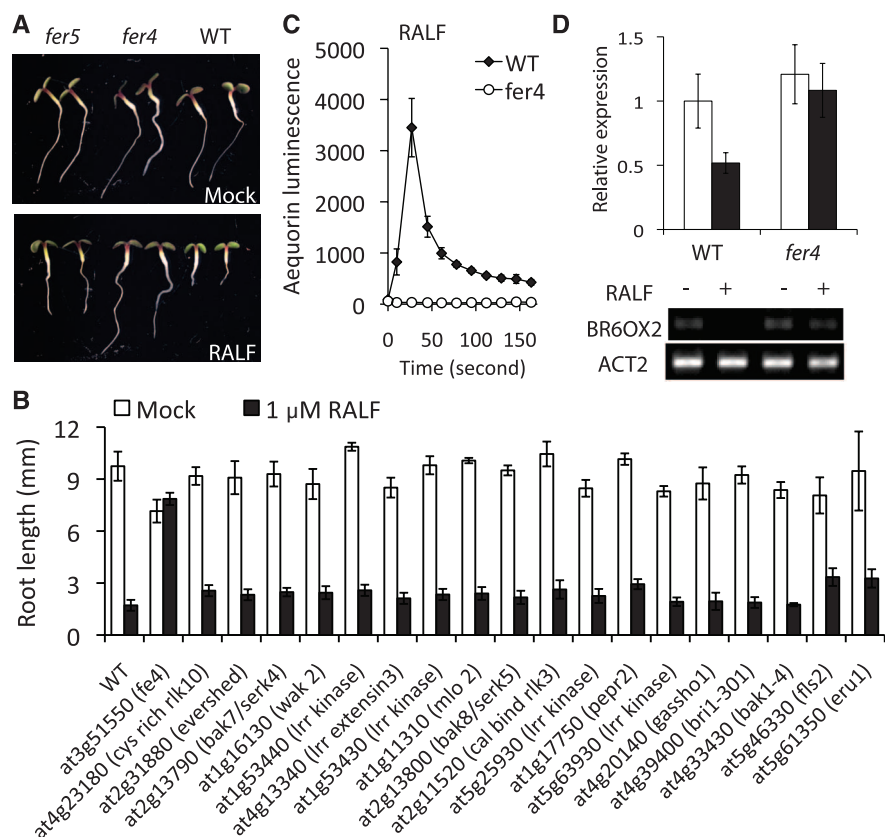


Fig. 2. Loss-of-function *fer* mutants are specifically insensitive to RALF. (A) The *fer4* and *fer5* plants show reduced sensitivity to RALF-induced root growth inhibition. Seedlings were treated with 1 μ M RALF. (B) Insensitivity of *fer* mutants to RALF is specific. Mutants of 18 other receptor-like proteins respond to RALF normally. (C) Normal FERONIA function is required for RALF-induced cytoplasmic calcium increase. Aequorin-expressing seedlings were treated with 500 nM RALF, $n = 6$. (D) RALF treatment causes a decrease of BR6OX2 expression in the wild type but not in the *fer4* mutant. ACT2 denotes actin. Error bars in (B), (C), and (D) denote SEM.

induced by RALF after 30 min of treatment (table S2). None of these other lines showed differences in RALF sensitivity of root elongation relative to the wild type (Fig. 2B), and thus we conclude that the *fer4* response to RALF is specific.

The above observations suggest that FERONIA mediates RALF's inhibitory effect on root elongation by inhibiting the activity of AHA2, which secretes protons into the apoplast (23). Our model predicts that the H^+ -ATPase activity is constitutively up-regulated in the *fer4* mutant; consistent with this prediction, experimental measurements showed that *fer4* mutants acidify the bathing media faster than the wild type (Fig. 3A). Moreover, in a root elongation assay in the presence of the inhibitory cation lithium, *fer4* mutant roots were hypersensitive and growth was poorer than in wild-type roots (Fig. 3B). This response is a typical phenotype of mutant plants containing increased H^+ -ATPase activity and a hyperpolarized plasma membrane, which causes increased uptake of the inhibitory cation into the cytoplasm (24). The effect of increased H^+ -ATPase activity on root growth was examined by growing seedlings under blue light, which promotes root elongation (25). The roots of both *fer4* and *ralf* loss-of-function mutants were longer than wild-type roots (Fig. 3C and fig. S16, A to D).

To test whether RALF binds to FERONIA, we performed coimmunoprecipitation of HisRALF or its inactive analog HisRALF(Δ 2-8) with hemagglutinin (HA)-tagged FERONIA (FER-HA) expressed in *Nicotiana benthamiana*. FER-HA protein, with an apparent molecular mass of ~140 kD, bound HisRALF (~8 kD; Fig. 4, A and B). His-RALF binding was greater than with the inactive analog by a factor of 4.5 to 6. The lack of effect of HisRALF(Δ 2-8) when added together with HisRALF in root inhibition assays indicates that HisRALF(Δ 2-8) lacks the ability to interact with the RALF receptor, and this was borne out by binding measurements (Fig. 4, A and C, and fig. S17A). In a separate experiment with 25 nM RALF labeled with 125 I, we measured the binding of RALF peptide to plasma membranes isolated from wild-type or *fer4* plants in the presence or absence of an excess amount (25 μ M) of nonradioactive RALF (Fig. 4D). Approximately half of the iodinated RALF bound to the wild-type plasma membrane was reduced by an excess amount of nonradioactive RALF, indicating that this portion of the binding is not only specific but also saturable. In the *fer4* mutant, the saturable binding was reduced by ~40% but not completely eliminated. The possibility that there are additional sites for RALF binding other than FERONIA in the plasma membrane of wild-type plants is supported by our observation that the *fer4* mutant is not completely insensitive to RALF at a higher concentration (5 μ M) in both the root growth inhibition test and cytoplasmic calcium assay (fig. S15, D and E). Direct and specific binding of RALF to FERONIA was further confirmed with in vitro binding assays using the ectodomain of FERONIA (ectoFER)

expressed as fusion proteins with either glutathione S-transferase (GST) or maltose binding protein (MBP) produced in *E. coli* (fig. S18, B to F). RALF specifically bound to GST-ectoFER as well as HisMBP-ectoFER; the inactive RALF analog showed little or no binding in pull-down assays with the ectoFER proteins (Fig. 4, E and F).

Our results show that FERONIA is a receptor for RALF and that this receptor kinase mediates RALF's inhibitory effects on the cell elongation

rate in *Arabidopsis* primary roots. Our demonstration that RALF is a naturally occurring ligand for FERONIA and that this kinase plays an important role in negatively regulating cell expansion provides an impetus for future work in evaluating the role that each RALF-like peptide plays in plant growth and development. The results also support the hypothesis that RALF, or another member of the 34 RALF-like peptides, is the ligand functioning in pollen tube arrest prior to fertilization.

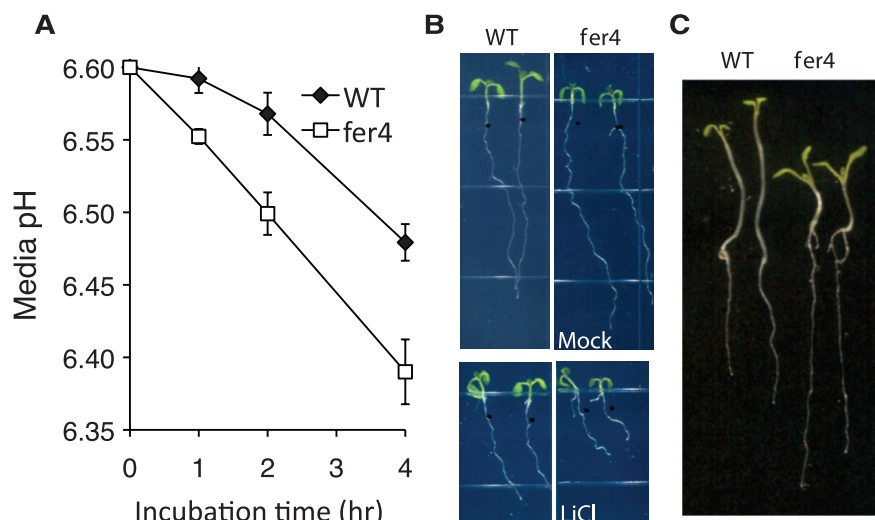
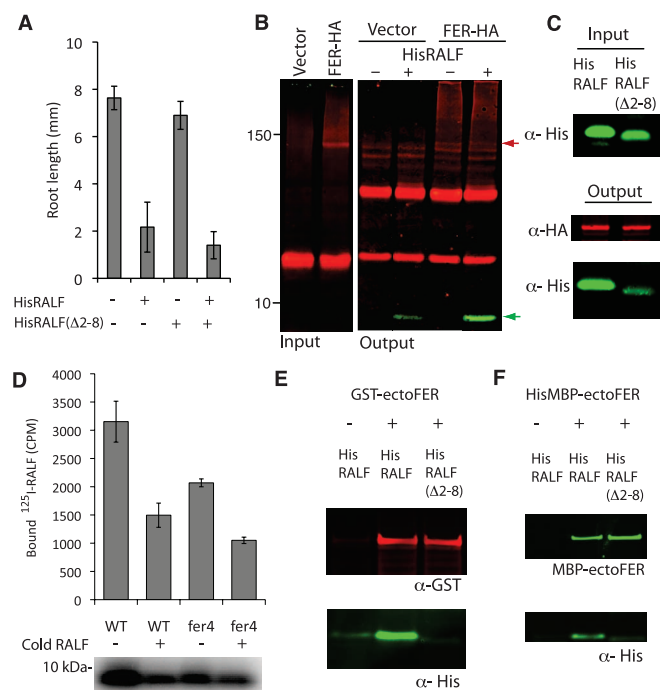


Fig. 3. Loss-of-function *fer* mutant exhibits the phenotype typical of plants with a higher plasma membrane H^+ -ATPase activity. (A) Seedlings of *fer4* mutant acidify bathing media faster than the wild type ($n = 7$ each). Error bars denote SEM. (B) The *fer4* root growth is hypersensitive to lithium ion stress, consistent with a hyperactive pump and a deeper membrane potential. (C) The *fer4* mutant shows longer root length. Seedlings were grown under blue light for 7 days.

Fig. 4. RALF binds to FERONIA. (A) HisRALF(Δ 2-8) is an inactive analog of RALF and does not compete with RALF in root growth inhibition assay. Data are means $\pm$ SD ($n = 6$). (B) RALF binds to the FERONIA protein expressed in tobacco. Red arrow indicates FER-HA; green arrow indicates HisRALF. (C) Binding of RALF to FERONIA is sequence-specific, and binding of the inactive analog HisRALF(Δ 2-8) to FERONIA is similar to background binding. Binding was immunodetected with antibodies to His tag (α -His) or HA (α -HA). (D) The *fer4* mutant plasma membrane shows reduced saturable binding of 125 I-RALF relative to the wild type. Data are means $\pm$ SE. Bound and released 125 I-RALF detected in protein gel shows reduced binding in the *fer4* mutant.



(E) HisRALF, but not HisRALF(Δ 2-8), binds to GST-tagged ectodomain of FERONIA receptor (GST-ectoFER). Data are representative of three experiments. (F) HisRALF, but not HisRALF(Δ 2-8), binds to HisMBP-tagged ectodomain of FERONIA (HisMBP-ectoFER).

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Acknowledgments: We thank H. Burch for technical assistance; M. Ivancic for advice on mass spectrometry data analyses; R. Wrobel, B. Fox (National Institute of General Medical Sciences Protein Structure Initiative, U54 GM074901), and S. Litscher for assistance with *E. coli* expression experiments; G. Barrett-Wilt for mass spectrometric

instrumentation; M. A. Beg, M. Hoffman, and O. Ginther for assistance with the iodination experiments; F. Li for advice on *Nicotiana* expression; S.-H. Su and the Plant Imaging Center for assistance with microscopy; and W. Aylward for advice on Etruscan mythology. Supported by NSF grant MCB-0929395 and U.S. Department of Energy Office of Basic Energy Sciences grant DEFG02-88ER13938 (M.R.S.), NSF grant DGE-1256259 (K.S.), and the National Human Genome Research Institute—University of Wisconsin Genomic Sciences Training Program (NIH grant 5T32HG002760, B.B.M.). Phosphoproteome data are deposited in PRIDE (Proteomics Identification Database) with accession numbers 31655 to 31656 and ProteomeXchange accession number PXD000515. Microarray data are deposited in ArrayExpress (E-MEXP-3994).

Supplementary Materials

www.sciencemag.org/content/343/6169/408/suppl/DC1
Materials and Methods
Figs. S1 to S14
Tables S1 and S2
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9 August 2013; accepted 17 December 2013
10.1126/science.1244454

A Different Form of Color Vision in Mantis Shrimp

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One of the most complex eyes in the animal kingdom can be found in species of stomatopod crustaceans (mantis shrimp), some of which have 12 different photoreceptor types, each sampling a narrow set of wavelengths ranging from deep ultraviolet to far red (300 to 720 nanometers) (1–3). Functionally, this chromatic complexity has presented a mystery (3–5). Why use 12 color channels when three or four are sufficient for fine color discrimination? Behavioral wavelength discrimination tests ($\Delta\lambda$ functions) in stomatopods revealed a surprisingly poor performance, ruling out color vision that makes use of the conventional color-opponent coding system (6–8). Instead, our experiments suggest that stomatopods use a previously unknown color vision system based on temporal signaling combined with scanning eye movements, enabling a type of color recognition rather than discrimination.

Stomatopods are benthic marine crustaceans that are generally found in tropical and temperate waters. Their compound eyes possess the largest number of photoreceptor types known in any animal [between 16 and 21 different receptors in some species (1, 3, 9)], allowing them to discriminate color (5) as well as both linear and circular polarized light (3, 10). Such retinal complexity is unrivaled in the animal kingdom, although papilionid butterflies may have up to eight spectral sensitivities (11). Theoretical approaches have predicted that between four and seven photoreceptor types are all that is needed to accurately encode the colors of the visible spectrum (12–14). The four-channel (tetrachromatic) solution that birds and reptiles use to sample a spectral range from 300 to 700 nm is optimally arranged to encode the known colors within this range. Where the spectrum examined loses the ultraviolet (UV) or red end, three photoreceptors

suffice, and trichromacy is the solution that many animals exhibit (12).

Our question was therefore, why do the stomatopods use 12 different photoreceptors to encode color? Before the experiments described here, Marshall *et al.* (5) demonstrated that stomatopods are capable of simple color discriminations based on color-card tests, similar to those devised by Carl von Frisch for bees and now used widely for

a number of animals (15). We hypothesized two alternative mechanisms for color information processing in stomatopods: (i) a multiple dichromatic color-opponent system (as described below), or (ii) the binning of colors into 12 separate channels, without any between-channel comparisons (4, 16).

Like butterflies, stomatopods have a variety of colorful body patterns, even using fluorescence to enhance color display (17). Furthermore, many of these species inhabit shallow coral reefs, one of the most colorful environments on Earth. The stomatopod's colors are thought to be involved in particularly complex communication systems, both between and within species (18), but little of this complexity requires a 12-dimensional color space to distinguish the colors available. Osorio *et al.* (19) speculated that stomatopods use their color sense to make reliable and quick judgements of color signals from conspecifics under changing light conditions, their steep-sided spectral sensitivities allowing particularly good color constancy. This would require comparison of the spectrally adjacent sharply tuned spectral sensitivities, and there is some anatomical evidence supporting this idea (6).

Stomatopod eyes are made up of a dorsal and ventral hemisphere, divided by a region of distinct

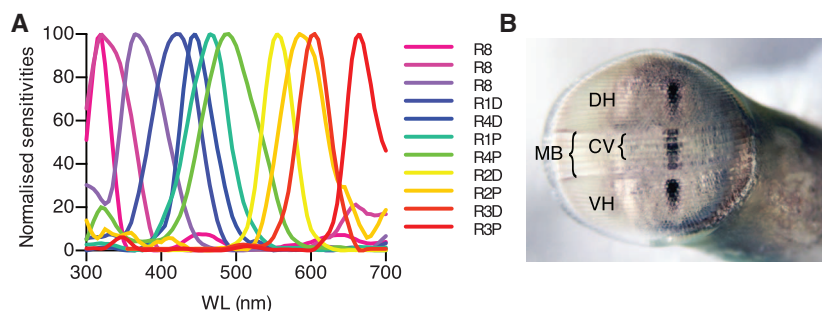


Fig. 1. (A) Spectral sensitivities of *H. trispinosa*. Spectral sensitivity curves obtained from intracellular electrophysiological recordings. The figure shows smoothed data (four neighbors on each side, second-order polynomial), normalized to 100% (see table S1). **(B) Eye of *H. trispinosa*.** Showing the dorsal hemisphere (DH) and ventral hemisphere (VH), divided by the midband (MB) containing the color receptors in the four top rows (CV).

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ommatidia (optical units) termed the midband (Fig. 1). Within two superfamilies of stomatopods (Gonodactyloidea and Lysiosquilloidea), this midband consists of six separate rows of ommatidia, each with different functionalities (2). Rows 1 to 4 are involved in color processing; rows 5 and 6 mediate the detection of circular or linear polarized light (3). A total of 12 cell types, each with different spectral sensitivities, are found within rows 1 to 4, with four UV-sensitive reticular cells located distally in the retina and by convention termed R8 cells (3). Beneath the R8 cells, the remaining seven reticular cells (R1 to R7) are further divided into two tiers (2). Comparison of the secondary R1 to R7 cell tiers would yield a set of highly tuned dichromatic mechanisms, sampling the 400- to 700-nm part of the spectrum in four bins as per hypothesis (i) above (3, 6, 19) (Fig. 1). Hypothesis (ii) would require a recognition of the pattern of excitation over the entire spectrum.

To distinguish between the two proposed hypotheses, we tested the ability of stomatopods to distinguish between different hues [spectral discrimination ($\Delta\lambda$) functions] using the Gonodactyloid stomatopod species *Haptosquilla trispinosa*. When two narrow-band spectral stimuli are presented simultaneously, they can only be discriminated when the difference between them is over a certain threshold, giving the minimum discriminable difference. Spectral discrimination curves obtained from other animals usually exhibit certain minima

in areas between the spectral sensitivity curves (20). Therefore, dichromats usually exhibit one such minimum, trichromats have two minima, tetrachromats have three, and so on. Our goal was therefore to test the color discrimination abilities of mantis shrimp by using a two-way choice test (Fig. 2 and fig. S1) in which the animal is trained to a specific wavelength by means of food rewards. The wavelength stimuli were presented to the animal with a pair of optical fibers, and a choice was recorded when the animal grabbed or tapped the end of the fiber. Test colors were presented together with the trained colors at varying wavelength intervals to determine at what point the animal could no longer discriminate between the two stimuli (i.e., when the success rate dropped to 50%).

Animals were trained successfully to 10 different color wavelengths: 400, 425, 450, 470, 500, 525, 570, 578, 628, and 650 nm. When the test wavelength was 50 to 100 nm from the trained wavelength, the success rates were between 70 and 80%, indicating that they discriminated well between the two stimuli. However, when the interval between the trained and test wavelengths was reduced to between 25 and 12 nm, the success rates dropped to around 50%, and it was clear that the stomatopods could no longer distinguish test from trained stimuli. An example of success rates is given in Fig. 2, and further results are shown in tables S2 and S3. The discrimination threshold was chosen to be at a 60% success rate, in accordance

with previous studies on animal discrimination thresholds (20). Using the interpolated points at 60%, we determined a relative spectral discrimination curve of $\Delta\lambda/\lambda$ (Fig. 3). The resulting values of $\Delta\lambda$ were all in the region between 12 and 25 nm, and the prominent dips, or minima, usually associated with the points between spectral sensitivity maxima in other studies were not clearly defined by spectral overlap regions (Fig. 3).

The potential spectral discrimination ability, based on previously known color vision systems, was also modeled to allow us to make predictions about the stomatopod color processing system. The

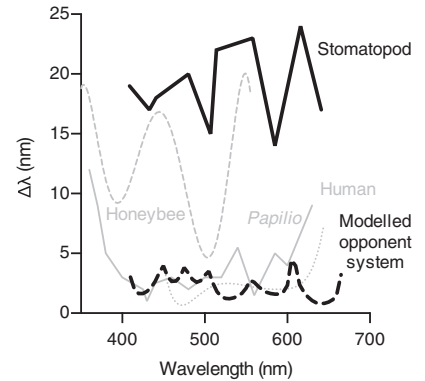


Fig. 3. Spectral discrimination curves ($\Delta\lambda/\lambda$). The spectral discrimination curve from behavioral testing of *H. trispinosa* is shown by a thick black line, and the modeled spectral discrimination curve is shown by a thick dashed line. [The figure is modified from Koshitaka *et al.* (20).]

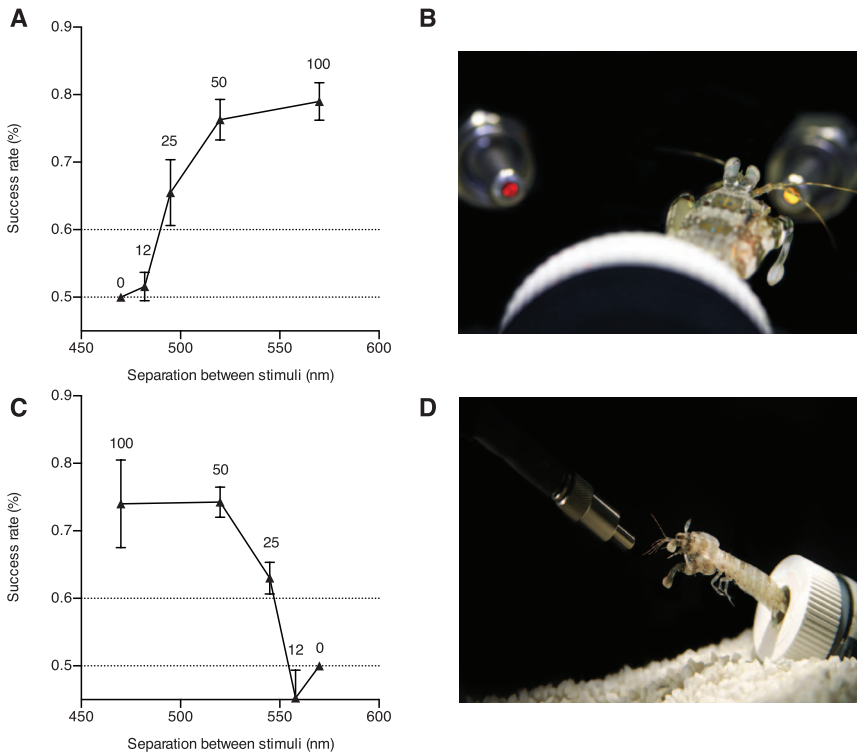


Fig. 2. Examples of correct choice data from a two-way choice test of *H. trispinosa*. Curves are plotted as mean $\pm$ SEM. The horizontal dashed lines indicate the 50% (chance) and 60% (discrimination) criteria. (A) Choices of animals trained to 470 nm ($n = 7$) and tested toward longer wavelengths. (C) Choices of animals trained to 570 nm ($n = 4$) and tested toward shorter wavelengths. The number above each point indicates the tested wavelength interval. (B and D) Examples of animals making a choice.

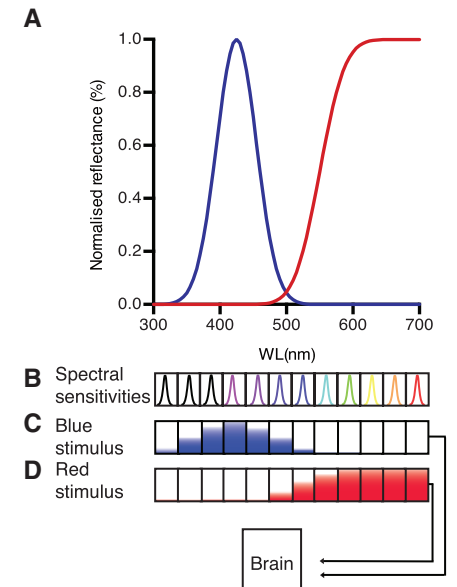


Fig. 4. Proposed processing mechanism. (A) Idealized spectral reflectance from stomatopod body parts. WL, wavelength. (B) Spectral sensitivities throughout the spectrum divided into separate bins. (C and D) Excitation patterns of each spectral sensitivity when looking at the blue (C) and red (D) reflectance spectra.

sensitivities of the photoreceptors in *H. trispinosa* were measured using intracellular electrophysiology (9) (table S1). Eight sensitivity maxima were found in the visible part of the spectrum, and another three were found in the UV {the fourth UV cell often proving hard to locate and record from [(9), Fig. 1]}. We modeled the stomatopod spectral discrimination curve using the Vorobyev/Osorio noise-limited model (21) (which determines color thresholds using photoreceptor noise levels) for a serial dichromatic system with comparison between each adjacent spectral sensitivity [mechanism (i) above (note S1)]. This system predicts very fine discrimination between 1 to 5 nm throughout the spectrum, with few peaks of coarser discrimination as seen in other animals. Such fine spectral discrimination would be expected in a color vision system that made analog comparisons between adjacent spectral sensitivities. The behavioral results presented here (Fig. 2) suggest that such analog comparisons are not made. Instead, stomatopod color vision is remarkably coarse (Fig. 3).

The results from our experiments suggest that the stomatopods do not use a processing system of multiple dichromatic comparisons as previously hypothesized based on assumed neural connections (16). Instead, we provide evidence that scanning eye movements (22) may generate a temporal signal for each spectral sensitivity, enabling them to recognize colors instead of discriminating them. (Fig. 4) (3, 4). In such a system, the 12 sensitivities (including the UV, not analyzed here but with its multiple sensitivities a good fit to the system envisaged) would be converted into a temporal pattern when scanned across an object, which the animal could recognize as color. This system is comparable to the spectral linear analyzers (termed "push-broom" analyzers because of the arrangement of the sensors and the flight direction) used in remote sensing systems (23) and is a unique way for animals to encode color. Although this system does not have the ability to discriminate between closely positioned wavelengths (and results in spectral "discrimination" defined by the distances between sensitivity peaks, seen when comparing Fig. 1 and 3), it would enable the stomatopod to make quick and reliable determinations of color, without the processing delay required for a multidimensional color space. Without the comparison of spectral channels, color constancy would not function in the way we currently understand it in other animals. Instead, identification of a color pattern by the mid-band and luminance by the hemispheres might function as a "panchromatic" method to discount illuminance (23). The eye is optically skewed so that both midband and hemispheres examine the same areas in space, which lends support to this idea (3). However, the details of the neural processing from the receptors remain unknown.

Stomatopods live a rapid-fire lifestyle of combat and territoriality, so possessing a simple, temporally efficient color recognition ability may be critical for survival (24, 25). As with many invertebrate information-encoding solutions, the actual processing of the problem is dealt with at

the periphery, in this case by an array of detectors seen in animals and unconsciously duplicated by remote-sensing engineers. What remains for us to discover is the nature of the information and its importance in the biological decisions these enginging crustaceans make.

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Acknowledgments: This work was supported by grants from the Asian Office of Aerospace Research and Development, the Air Force Office of Scientific Research, the Australian Research Council, the Lizard Island Research Foundation, and a Doctoral Fellowship (2013) from the Lizard Island Research Station, a facility of the Australian Museum. We thank the reviewers for their great comments and W.-S. Chung, M. Bue, and R. Bedford for technical help. All data described in this manuscript are presented in either the main manuscript or the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/343/6169/411/suppl/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 to S3
References (26–29)

11 September 2013; accepted 19 November 2013
10.1126/science.1245824

Risky Ripples Allow Bats and Frogs to Eavesdrop on a Multisensory Sexual Display

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Animal displays are often perceived by intended and unintended receivers in more than one sensory system. In addition, cues that are an incidental consequence of signal production can also be perceived by different receivers, even when the receivers use different sensory systems to perceive them. Here we show that the vocal responses of male túngara frogs (*Physalaemus pustulosus*) increase twofold when call-induced water ripples are added to the acoustic component of a rival's call. Hunting bats (*Trachops cirrhosus*) can echolocate this signal by-product and prefer to attack model frogs when ripples are added to the acoustic component of the call. This study illustrates how the perception of a signal by-product by intended and unintended receivers through different sensory systems generates both costs and benefits for the signaler.

Elaborate courtship displays are favored by sexual selection but are often opposed by predation (1, 2). Animals can produce complex signals that are detected and processed through multiple sensory systems [commonly referred to as multimodal or multisensory signals (3, 4)]. Many communication systems once thought to operate primarily in a single sensory mode actually include secondary components that stimulate additional

senses (5–7). For instance, lip movements are necessary to produce human speech, but the associated visual cue of moving lips can also have a dramatic impact on speech perception (7). Such secondary signal components can be beneficial when they enhance the detection and perception of signals (3, 4). However, communication between the sender and intended receiver rarely occurs in private channels (8), and signalers are prone to costs imposed by eavesdroppers, such as predators and parasites who also attend to their displays (9–11).

Many male frogs possess conspicuous vocal sacs that evolved to recycle air during the production of their advertisement calls (6). The inflation and deflation of the vocal sac additionally act as

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visual cues in female attraction and male competition (12–14). Furthermore, when frogs call from the water's surface, their body movement incidentally creates surface waves or ripples which may be perceived as a third component by receivers (a tactile component in addition to the acoustic and visual components (15) (Fig. 1 and movie S1). Water ripples play a communicative role in some frog species (16, 17), but we have had no understanding of how call-induced cues are integrated with the perception of the acoustic component of the call by intended receivers and eavesdroppers.

In our study system, male túngara frogs (*Physalaemus pustulosus*) aggregate at night in shallow ponds and call to attract females (18). Males compete acoustically with other túngara males and maintain spacing by physically defending a calling site with a radius of roughly 7.5 cm against competitors (18). Males display from shallow puddles and create ripples as a by-product of call production, which can potentially be used by other males to assess the location of rivals. The frog-eating bat (*Trachops cirrhosus*) can eavesdrop on the acoustic component of the frog's call (11, 19). To reduce predation risk, frogs stop calling in response to predator cues (20), a strategy that has been shown to effectively increase localization errors by bats (21). Other bat species are able to use echolocation to detect small water droplets released into the air (splashes) or other cues produced when aquatic prey disrupt the water surface (22–24). We hypothesized that ripples produced during calling may aid frog-eating bats in the detection or localization of calling frogs, reducing the effectiveness of the frog's antipredator strategy of call cessation (20, 21).

Male túngara frogs generally show one of three behaviors in response to a nearby calling male, which is probably related to the risk of aggressive escalation and the motivation to fight (2). A challenged male can deflate his vocal sac and cease calling (but remain stationary at his call site), increase his call rate, or approach the rival to fight (18). We tested whether males used ripples associated with calling to assess rival competition. In playback experiments, males were presented with a multisensory display (sound plus ripples), sound alone, and ripples alone. We also varied the distance from the stimulus to the male [inside versus outside the physically defended range; (25) and fig. S1].

All males responded to ripples when they co-occurred with the acoustic signal (Fig. 1). On average, the call rate more than doubled during exposure to the multisensory signal as compared to the rate with sound alone [generalized linear mixed model (GLMM); $n = 22$ frogs, $df = 21$, t statistic (t) = 6.87, $P < 0.001$; Fig. 1 and movie S2]. Males did not respond to ripples alone, demonstrating that sound is the dominant signal component of the courtship behavior.

Male responses to ripples were dependent on distance. When the stimulus was within 7.5 cm of the focal male (i.e., within the zone that is physically defended), males called less in response to sound plus ripples and occasionally

ceased calling and deflated their vocal sac. When the stimulus was broadcast from 30 cm away, however, males increased their rate of response when ripples accompanied sound (GLMM, effect of distance on call cessation: $n = 18$ frogs, $df = 1$, $\chi^2 = 6.05$, $P = 0.014$; effect on call rate: $n = 18$ frogs, $df = 17$, t value = 5.79, $P < 0.001$; Fig. 1). Distance from the playback source, however, did not affect male response to only the acoustic component of the signal (call cessation: $n = 16$ frogs, $df = 1$, $\chi^2 = 0.0$, $P = 1.0$; call rate: $n = 14$ frogs, $df = 13$, t value = 1.46, $P = 0.17$).

The ripples produced during calling travel slowly in shallow water (~25 cm/s) (26) and therefore could potentially be detected by an eavesdropping predator for up to several seconds after call cessation. We tested whether bats could make use of such an aftereffect by offering bats ($n = 10$) a choice between a frog model next to a pool in which we generated the acoustic component of the call and ripples or a model that was placed next to a control pool in which the acoustic component of the call was broadcast but which lacked ripples [(25) and fig. S1]. In each trial, both stimuli were presented simultaneously and both were immediately halted when the bat flew from its perch, thereby mimicking the natural antipredator response of the frogs. Although sound immediately ceased at this point, ripple propagation continued

until the ripples reached the end of the experimental pool 2 to 3 s later.

Bats preferentially attacked the model associated with ripples (GLMM; $df = 1$, z score (z) = 2.81, $P = 0.005$; Fig. 2 and movie S3). The attack rate on the multisensory signal (sound plus ripples) increased by 36.5% as compared to the control signal (sound alone). These experiments demonstrate that bats make use of call-induced ripples, most likely through echolocation, because the experiments were conducted with no visible light and thus should have deprived the bat of visual cues.

We assessed the detection limits of echoes returning from smooth versus disturbed water surfaces by broadcasting an artificial sonar call to the experimental pools under varying angles and recording the returning echoes. Echo amplitude, and hence signal detection, strongly depends on the angle between sonar beam and water surface because of the acoustic mirror effect (24), making it unlikely that bats perceived the ripples from their perch (see also echo examples in Fig. 2). At close range, echoes returning from pools with ripples had higher variance in amplitude than echoes from control pools with smooth water surfaces (Fig. 2), generating a detectable cue for bats as they approach.

In nature, the túngara frogs' calling sites vary in the amount of clutter on or near the water surface,

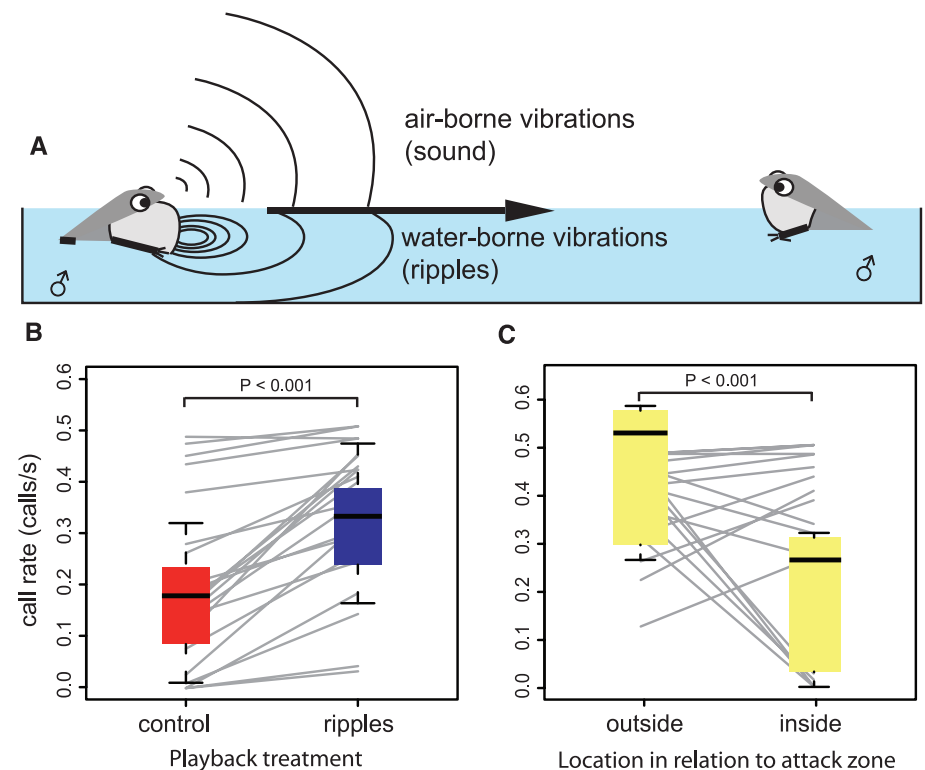


Fig. 1. Call-induced water ripples alter rival responses. (A) Calling male túngara frogs produce airborne vibrations (acoustic sound), the primary signal component, known to target males and females. Males also create waterborne vibrations (water ripples), an incidental by-product of calling from water surfaces. (B) Males increase call rates in response when ripples are added to sound playback. (C) When ripples and sound are played simultaneously from within the physically defended zone (7.5 cm), a significant proportion of males reduce their call rate, cease calling, and/or deflate, presumably to fight or flee. Shown are box plots of male call rate model estimates plus the individual responses of tested frogs (gray lines).

ranging from completely open to completely vegetated. We varied the calling environment by placing screens partially covered with leaf litter over the two experimental pools (a treatment that increases echolocation clutter) and presented bats with stimuli emanating from sites with and without clutter. Clutter treatment had a strong effect on attack preference ($df = 1$, $\chi^2 = 16.02$, $P = 0.005$, Fig. 2): When both pools were partially covered with leaf litter, the attack preference disappeared ($df = 1$, $z = 0.34$, $P > 0.97$).

Sexual selection has been responsible for generating some of the most elaborate behavioral phenotypes in the animal kingdom (1), but the evolution of such traits may be restricted by an increased risk of eavesdropping by parasites and

predators (3, 9). Understanding the specific mechanisms responsible for driving the diversification of multisensory signals is challenging. It requires demonstration of the effects of individual and combined signal components on receivers (e.g., how receivers perceptually integrate multiple signal components), and it requires an understanding of costs imposed on those signals. Our results provide three important insights into this process. First, we demonstrated that male túngara frogs attend to ripples in addition to the calls of perceived rivals. This probably improves the ability of males to make decisions about calling effort and risk relative to competition from nearby conspecifics (27). Ripples may also aid females in localization of the signaler through their integra-

tion with airborne sound (28). Second, we showed that the same signal by-product can be detected through different sensory systems by intended and unintended receivers. Detection of ripples by túngara frogs probably occurs via tactile stimulation, whereas bats detect them in the acoustic domain through echolocation. The perception of signal by-products through different senses by different receivers increases the range of eavesdroppers that can exploit the communication system. Third, predator detection of these signal by-products imposes a strong cost on signalers because of the frog's inability to halt ripple propagation (see movie S3 for a demonstration of a bat circling the ripples at some distance from the source, ~2 s after we stopped call production). This could counter the frog's main antipredator strategy of call cessation, because ripples leave a "footprint" of the frog's presence for several seconds after the male has disappeared from the acoustic channel. All signals and associated by-products produce disturbances in the environment, which can be perceived by multiple receiver species in different sensory systems, exposing the signalers to a complex array of costs and benefits.

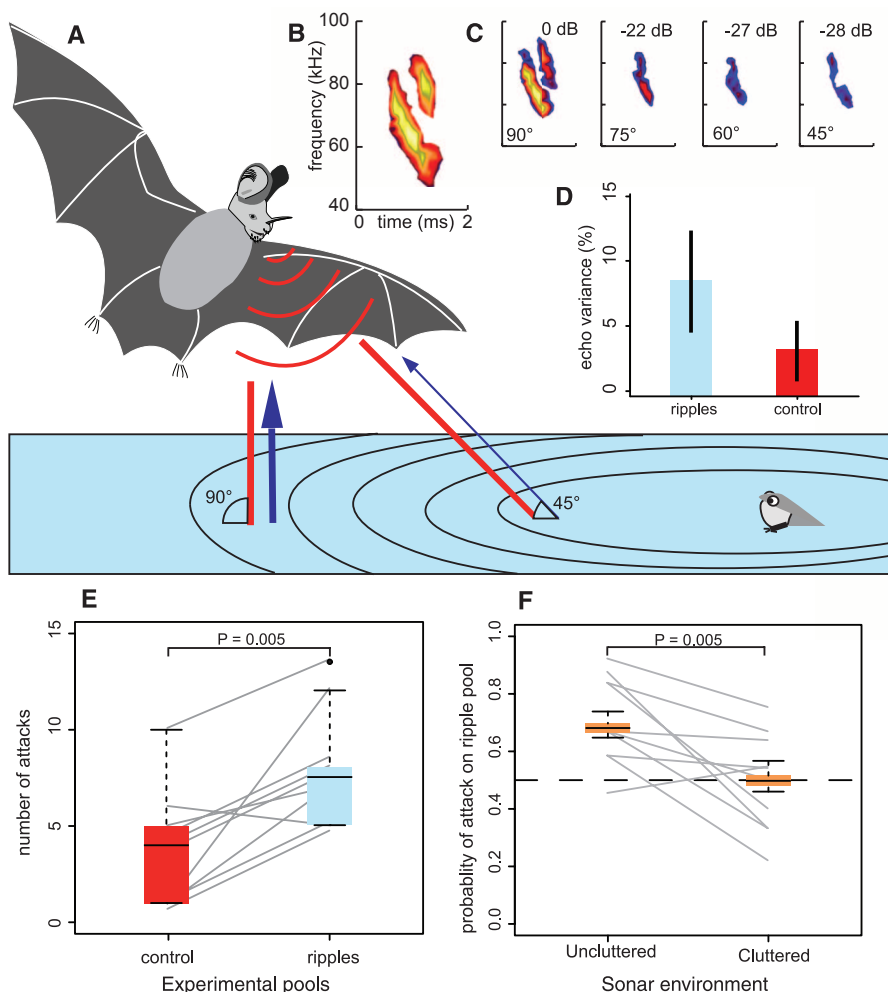


Fig. 2. Bats use call-induced ripples to hunt frogs. (A) Frog-eating bats rely partially on echolocation to hunt their prey. Water surfaces are highly reflective of echolocation signals, but the amount of returning echo (blue arrows) depends strongly on the angle between the signal propagation path (red lines) and the surface. (B) Spectrographic example of a bat's echolocation signal, with time on the x axis and frequency on the y axis. (C) Echo images derived from broadcasting a synthetic sonar call under different angles to the test pool. Echo amplitude as well as overall structure quickly decreased with decreasing angles. (D) Disturbed water surfaces during ripple playback produce higher variance in echo amplitude than do the smooth surfaces. (E) Results from a two-choice test with bats, showing a preference to attack the ripple pool over the control pool. (F) Ripple preference depends on environmental conditions. When both pools were covered with a layer of leaves (cluttered environment), preference disappeared. Graphs show mean $\pm$ SD (D) or box plots of model estimates + individual lines [(E) and (F)].

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Acknowledgments: We are grateful to M. Dixon, A. Lea, K. Ottens, J. Jacobitz, J. Finley, F. Rhebergen, and M. Shuman-Goodier for valuable help during the experiments. Comments from D. Blumstein, H. Slabbekoorn, J. Ratcliffe, and one anonymous reviewer substantially improved the manuscript, and J. Touchon and S. Dennis provided statistical advice. We thank the late Stan Rand for illuminating discussions about ripples in communication. We thank B. Klein, P. Clements, and Moey Inc. for fabricating the pneumatic robotic frog system. The research was funded

through a Rubicon grant (825.11.026) to W.H. from the Netherlands Organization for Scientific Research; an NSF grant (IOS 1120031) to R.C.T., M.J.R., and R.A.P.; and resources made available to R.A.P. from the Smithsonian Tropical Research Institute (STRI). All research reported here complied with STRI Institutional Animal Care and Use Committee protocols. We obtained all required permits from the government of Panama. The authors report no conflict of interest. Raw data will be made available at the Dryad Data Repository (10.5061/dryad.82t1k).

Supplementary Materials

www.sciencemag.org/content/343/6169/413/suppl/DC1
Materials and Methods
Fig. S1
References (29–33)
Movies S1 to S3

16 August 2013; accepted 20 November 2013
10.1126/science.1244812

Endothelial Cell-Derived Angiopoietin-2 Controls Liver Regeneration as a Spatiotemporal Rheostat

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Liver regeneration requires spatially and temporally precisely coordinated proliferation of the two major hepatic cell populations, hepatocytes and liver sinusoidal endothelial cells (LSECs), to reconstitute liver structure and function. The underlying mechanisms of this complex molecular cross-talk remain elusive. Here, we show that the expression of Angiopoietin-2 (Ang2) in LSECs is dynamically regulated after partial hepatectomy. During the early inductive phase of liver regeneration, Ang2 down-regulation leads to reduced LSEC transforming growth factor- β 1 production, enabling hepatocyte proliferation by releasing an angiocrine proliferative brake. During the later angiogenic phase of liver regeneration, recovery of endothelial Ang2 expression enables regenerative angiogenesis by controlling LSEC vascular endothelial growth factor receptor 2 expression. The data establish LSECs as a dynamic rheostat of liver regeneration, spatiotemporally orchestrating hepatocyte and LSEC proliferation through angiocrine- and autocrine-acting Ang2, respectively.

The vascular endothelium is considered a passive cell population that acts in response to exogenous cytokines. However, recent work has shown that the endothelium can actively function as gatekeeper of tissue homeostasis. Endothelial cell-derived angiocrine signals orchestrate organogenesis during development (1, 2) and promote liver and lung regeneration in the adult (3, 4). Liver regeneration is a prototypic example of the intricate cross-talk between parenchymal cells and stromal cells (5–8). Liver sinusoidal endothelial cells (LSECs) have been shown to exert protective functions on hepatocytes (9) and promote hepatocyte proliferation during liver regeneration (3).

In order to systematically analyze the mechanisms of LSEC-regulated angiocrine growth con-

trol during liver regeneration, we isolated LSEC from sham-operated and two-thirds partial hepatectomized mice 1 day after surgery and performed transcriptomic gene expression analyses. Ninety-three genes were significantly up-regulated in LSEC upon partial hepatectomy (PHx) (table S1). Only nine genes were significantly down-regulated. Among the most strongly down-regulated LSEC genes was Angiopoietin-2 (Ang2) (Fig. 1A and table S2). Ang2 is a contextual antagonist of

the vascular receptor tyrosine kinase Tie2 and is expressed at low levels in resting endothelial cells (10, 11). Angiogenic or inflammatory endothelial activation leads to the up-regulation of Ang2 (12–14). The rapid down-regulation of LSEC Ang2 after PHx was consequently counterintuitive and prompted us to systematically study the role of LSEC-derived Ang2 during liver regeneration.

Quantitative polymerase chain reaction (PCR) analysis of Ang2 in liver lysates confirmed the rapid Ang2 down-regulation after PHx. One day after PHx, Ang2 mRNA levels were down-regulated to 18% of liver lysates from sham-operated mice. Ang2 expression thereafter steadily recovered to normal levels at day 8, when the liver restores its normal mass (Fig. 1B). The temporal pattern of Ang2 expression corresponded to the well-established pattern of hepatocyte and nonparenchymal cell proliferation after PHx (15). In mice, liver regeneration after PHx occurs rapidly by means of hepatocyte hyperplasia and hypertrophy to reach a proliferation peak as early as 48 hours after hepatectomy (16). Thereafter, hepatocyte proliferation steadily declines to baseline (15). In contrast, nonparenchymal cells, including LSECs, reach a proliferation peak 4 days after PHx, which is concomitant with the gradual recovery of Ang2. We therefore hypothesized that LSEC-derived Ang2 may negatively control hepatocyte proliferation and that Ang2 down-regulation after PHx may contribute to hepatocyte proliferation by releasing an angiocrine growth regulatory brake.

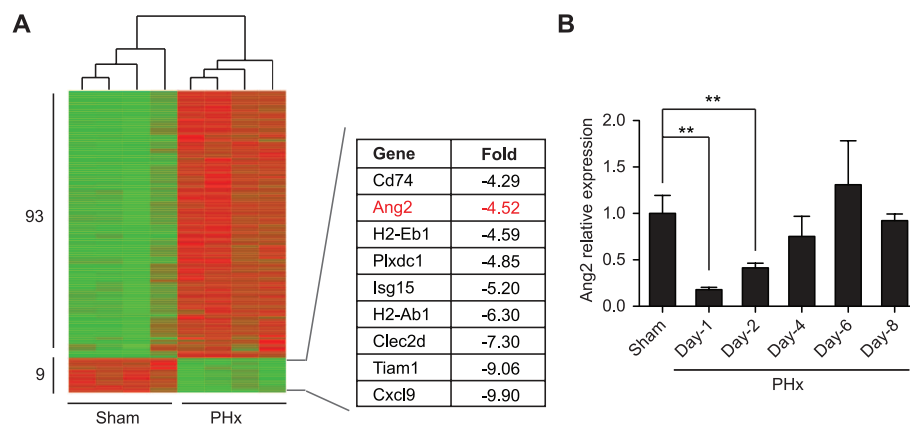


Fig. 1. Dynamics of Ang2 expression in LSECs after hepatectomy. (A) Heat map representation of significantly changed LSEC genes from sham operated and hepatectomized mice one day after surgery ($n = 4$ mice). Details of identified genes are listed in tables S1 and S2. **(B)** Temporal kinetics of Ang2 expression during liver regeneration by means of quantitative PCR analysis of mRNA from whole-liver lysate (mean $\pm$ SD, $n = 4$ mice, $**P < 0.01$).

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To examine this hypothesis, we performed PHx in wild-type (WT) and Ang2-deficient mice. Although Ang2-deficient mice showed impaired

postnatal retinal angiogenesis characterized by a chaotic and incomplete vascular plexus (fig. S1) (17), the mice developed normally and had a

similar life span as that of WT mice. Livers of Ang2-deficient mice were normal in size and showed the same hierarchical macrovasculature

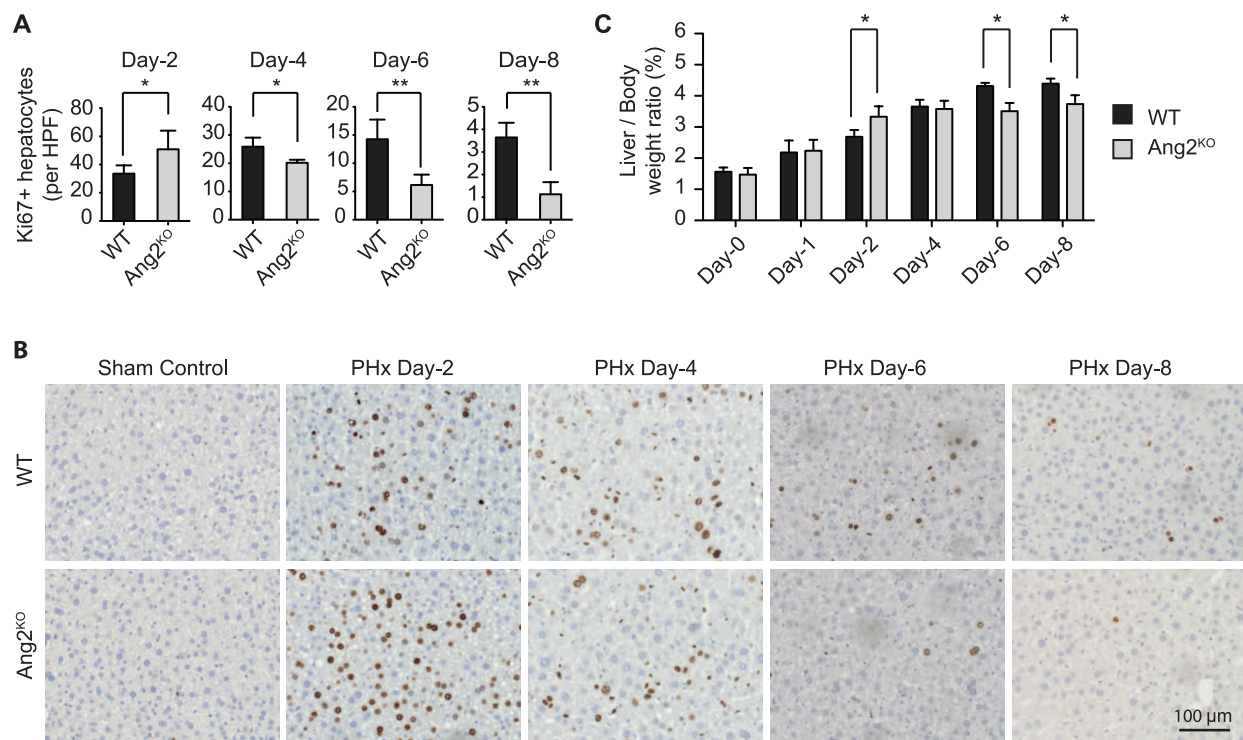


Fig. 2. Genetic ablation of Ang2 dynamically alters hepatocyte proliferation during liver regeneration. (A) Quantitation of Ki67-positive proliferating hepatocytes in liver sections of WT and Ang2-deficient mice after PHx

(mean $\pm$ SD, $n = 4$ to 6 mice, $*P < 0.05$, $**P < 0.01$). (B) Representative images of Ki67-positive hepatocytes (brown stained large nuclei). (C) Liver-to-body-weight ratios of WT and Ang2-deficient mice after PHx (mean $\pm$ SD, $n = 4$ to 6 mice, $*P < 0.05$).

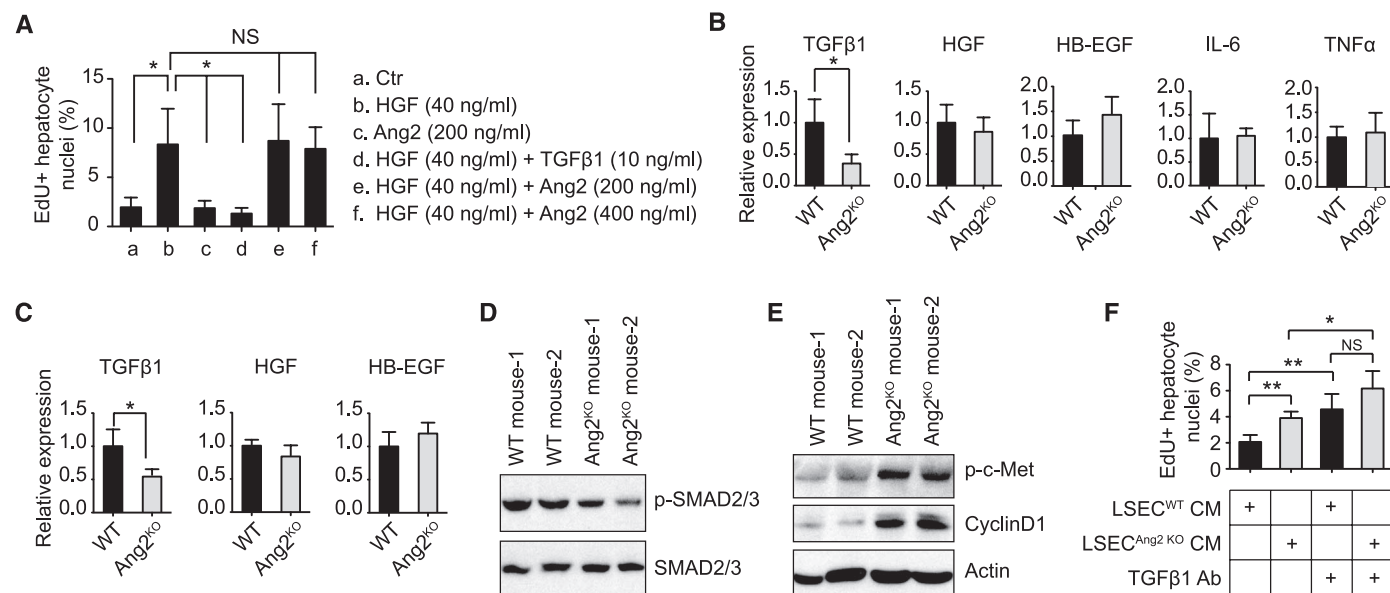


Fig. 3. LSEC-derived Ang2 regulates hepatocyte proliferation indirectly by modulating the expression of TGFβ1 in LSEC. (A) Primary WT mouse hepatocytes were stimulated with different cytokines and the percentage of EdU⁺ hepatocytes was determined (mean $\pm$ SD, $n = 3$ mice, $*P < 0.05$) (fig. S7, representative images). (B) TGFβ1, HGF, HB-EGF, IL-6, and TNFα mRNA expression was measured with quantitative PCR of liver lysates 1 day after PHx (mean $\pm$ SD, $n = 4$ mice, $*P < 0.05$). (C) TGFβ1, HGF, and HB-EGF expression was quantified with quantitative PCR of freshly isolated LSEC

1 day after PHx (mean $\pm$ SD, $n = 4$ mice, $*P < 0.05$). (D and E) Protein levels and phosphorylation states of SMAD, c-MET, and cyclin D1 in liver lysates of WT and Ang2-deficient mice were analyzed with immunoblotting. (F) Primary WT mouse hepatocytes were stimulated with conditioned medium from WT or Ang2-deficient LSECs supplemented with or without TGFβ1 neutralizing antibody, hepatocytes were pulsed with EdU, and the percentage of EdU⁺ hepatocytes was determined (mean $\pm$ SD, $n = 4$ mice, $*P < 0.05$, $**P < 0.01$).

and microarchitecture of liver sinusoids as seen in WT mice (figs. S2 and S3 and movies S1 and S2). Two days after PHx at the peak of hepatocyte proliferation, liver regeneration in Ang2-deficient mice was enhanced as evidenced by significantly more Ki67⁺ hepatocytes and larger liver mass as compared with that of WT mice (Fig. 2, A to C). This pattern of increased hepatocyte proliferation was reverted during the angiogenic phase (days 4 to 8). In contrast to the steady increase of liver mass in WT mice, hepatocyte proliferation was lower in Ang2-deficient mice during the angiogenic phase of liver regeneration (Fig. 2, A to C). Similar results were obtained in WT mice treated with Ang2-neutralizing antibody (fig. S4, A to D).

To unravel the mechanism of Ang2-regulated hepatocyte proliferation, we examined possible direct effects of Ang2 on hepatocytes. First, the exclusive expression of Ang2 and its receptor Tie2 in LSEC was confirmed (figs. S5, A and B, and S6A). Next, we directly stimulated hepatocytes with hepatocyte growth factor (HGF) and Ang2. In contrast to HGF, which induced strong c-Met, AKT, signal transducers and activators of transcription 3 (STAT3), c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK) phosphorylation, Ang2 failed to trigger any intrinsic signaling in hepatocytes (fig. S5, C and D). Moreover, EdU incorporation revealed no direct effect of Ang2 on hepatocyte DNA synthesis (Fig. 3A and fig. S7).

To identify Ang2-regulated LSEC-derived hepatotropic cytokines, we performed a candidate-based screen of established hepatocyte growth factors. Expression of HGF, tumor necrosis factor- α (TNF α), heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF), and interleukin-6 (IL-6) was not altered in livers of Ang2-deficient mice 1 day after PHx. In contrast, expression of the potent inhibitor of hepatocyte proliferation transforming growth factor- β 1 (TGF β 1) was reduced in Ang2-deficient mice (Fig. 3B). The detailed temporal analysis of Ang2 and TGF β 1 expression identified a close relationship between Ang2 and TGF β 1 (fig. S8).

To validate the cellular sources of the analyzed cytokines, we examined the differential cytokine expression pattern in freshly isolated hepatic cells, including hepatocytes, LSECs, Kupffer cells, and stellate cells. TGF β 1 and HB-EGF were predominately expressed by LSECs. On the contrary, HGF, IL-6, TGF β 2, and TGF β 3 were primarily expressed by stellate cells (fig. S6B) (18–21). Consistent with the TGF β 1 expression in whole liver lysates, TGF β 1 expression in LSECs of Ang2-deficient mice decreased by 50% compared with that of WT LSECs (Fig. 3C). Enzyme-linked immunosorbent assay analyses of liver lysates from hepatectomized mice similarly showed reduced TGF β 1 levels in Ang2-deficient mice (fig. S9A).

TGF β 1 controls hepatocyte proliferation by binding to its type II receptor (TGFBR2) and then recruiting type I receptor (TGFBR1) (22). This activates downstream SMAD signaling, leading to reduced Cyclin D1 and Cyclin E ex-

pression and hepatocyte cell cycle arrest (23). Although the expression of TGFBR1 and TGFBR2 was rapidly down-regulated after PHx (fig. S9B) (24), their expression was similar in hepatectomized livers of WT and Ang2-deficient mice (fig. S9C). Corresponding to the reduced TGF β 1 in Ang2-deficient mice, SMAD2 and SMAD3 phosphorylation in liver lysates of Ang2-deficient mice was significantly reduced as compared with WT mice (Fig. 3D). Serum HGF levels were similar in WT and Ang2-deficient mice (fig. S10). Yet, Ang2-deficient mice showed stronger phosphorylation of c-Met in the liver (Fig. 3E). In line with reduced TGF β 1-TGFBR activity, Cyclin D1 expression levels were significantly elevated in Ang2-deficient livers after PHx (Fig. 3E).

To validate the LSEC-controlled Ang2/TGF β 1 hepatocytes growth regulatory loop, we stimulated cultured hepatocytes with conditioned media from WT or Ang2-deficient LSECs. Hepatocyte exposed to conditioned medium from Ang2-deficient LSEC showed significantly higher EdU incorporation. Addition of a TGF β 1-neutralizing antibody further increased EdU incorporation

(Fig. 3F), validating that Ang2 indirectly regulated hepatocyte proliferation by controlling LSEC-derived TGF β 1 expression.

In contrast to enhanced liver regeneration in Ang2-deficient mice during the early inductive phase (days 0 to 3), liver regeneration was significantly impaired in Ang2-deficient mice at later stages (days 4 to 8) (Fig. 2A). Cleaved Caspase-3 staining revealed that hepatocyte apoptosis was a rare event during later stages of liver regeneration (fig. S11). The later angiogenic phase of liver regeneration after PHx (days 4 to 8) is characterized by expansion of the nonparenchymal compartment (LSECs, Kupffer cells, and stellate cells) at a time when most hepatocyte proliferation is completed (15). We consequently hypothesized that the impaired liver regeneration in Ang2-deficient mice during the later angiogenic phase could have resulted from changes in stromal cell proliferation. Indeed, Ang2-deficient mice showed a significant reduction of nonparenchymal cell proliferation (fig. S12). To further distinguish which nonparenchymal cell population was affected, mice were pulsed for 2 hours with EdU 4

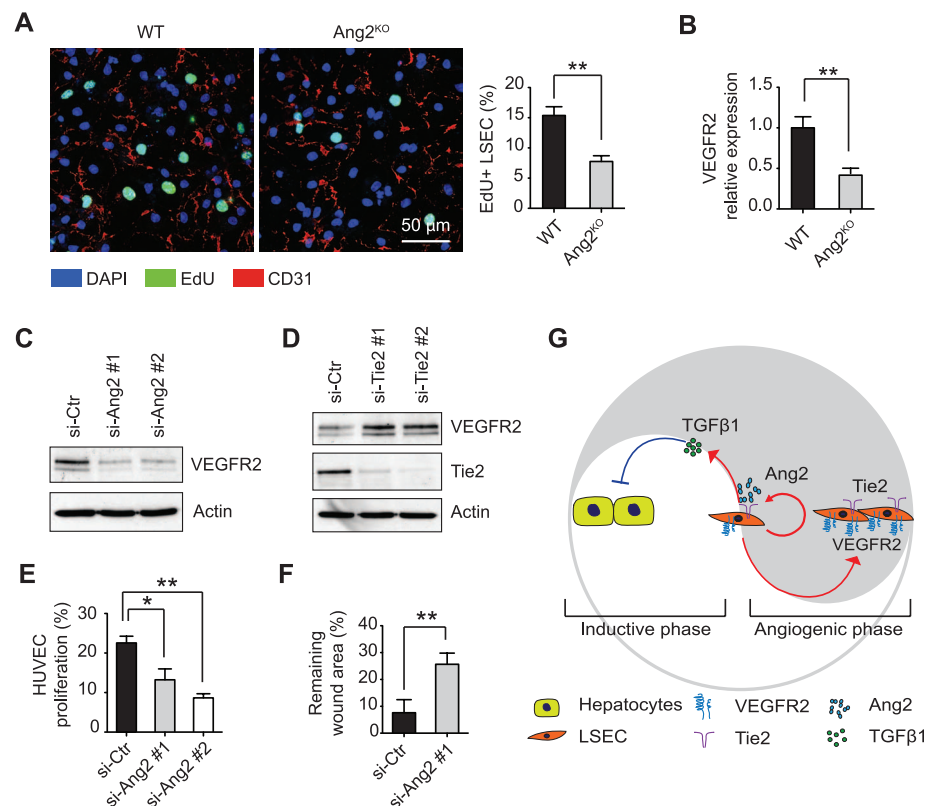


Fig. 4. Ang2 deficiency inhibits LSEC proliferation and contributes to impaired liver regeneration during the angiogenic phase of liver regeneration. (A) Four days after PHx, WT and Ang2-deficient mice were pulsed with EdU, and the percentage of proliferating LSEC was quantified (mean $\pm$ SD, $n = 3$ mice, $**P < 0.01$). (B) VEGFR2 mRNA of LSECs isolated from WT and Ang2-deficient mice 4 days after hepatectomy was quantified with quantitative PCR (mean $\pm$ SD, $n = 3$ mice, $**P < 0.01$). (C and D) VEGFR2 levels in control small interfering RNA (si-CTR), Ang2 siRNA (si-Ang2), or Tie2 siRNA (si-Tie2)-transfected HUVEC were analyzed by means of immunoblotting. (E) Proliferation of si-CTR- and si-Ang2-transfected HUVEC was quantified by means of EdU incorporation assay (mean $\pm$ SD, $n = 3$ replicates, $*P < 0.05$, $**P < 0.01$) (fig. S16, representative images). (F) Migration of HUVEC after Ang2 knockdown was evaluated by means of wound healing assay (mean $\pm$ SD, $n = 4$ replicates, $**P < 0.01$) (fig. S17, representative images). (G) Model of endothelial-derived Ang2 function in the control of liver regeneration.

days after PHx. Subsequently, nonparenchymal cells were isolated, immunostained, and analyzed. This analysis revealed that LSEC proliferation was dramatically reduced in the absence of Ang2 (Fig. 4A). Hepatocyte-derived vascular endothelial growth factor (VEGF) is known to act as a potent stimulus for LSEC proliferation (25). However, serum VEGF levels were similar in WT and Ang2-deficient mice 2 and 4 days after PHx (fig. S13). Candidate-based gene expression screening revealed that VEGF receptor 2 (VEGFR2) expression in LSEC was decreased by 60% in Ang2-deficient mice (Fig. 4B). Expression of the transcription factor Id1, which acts downstream of VEGFR2, was unchanged in the absence of Ang2 (fig. S14). However, the expression of Wnt2, a hepatotropic angiocrine factor whose expression is controlled by VEGFR2 signaling (3), was markedly reduced in Ang2-deficient LSECs during the angiogenic phase (fig. S14).

We further validated that Ang2 regulated VEGFR2 expression in cultured human endothelial cells (HUVECs). Ang2 silencing significantly reduced mRNA and protein levels of VEGFR2 (Fig. 4C and fig. S15A). Moreover, Ang2 overexpression led to increased VEGFR2 expression (fig. S15B). Conversely, silencing of the Ang2 receptor Tie2 increased VEGFR2 expression, indicating that Ang2-regulated VEGFR2 expression was Tie2-dependent (Fig. 4D). In line with the Ang2-regulated VEGFR2 expression, Ang2 silencing not only reduced HUVEC proliferation but also inhibited HUVEC migration and delayed wound closure (Fig. 4, E and F, and figs. S16 and S17). Consistently, migration of cultured LSEC from Ang2-deficient mice was similarly impaired (fig. S18).

This study was aimed at elucidating angiocrine mechanisms of liver regeneration. After the identification of Ang2 as a key vascular-derived regulator of liver regeneration, we hypothesized that similar paracrine cross-talk mechanisms may be operative during chronic liver injury. To study this hypothesis, WT and Ang2-deficient mice were treated with CCL₄. Chronic liver injury was evident by a rough liver surface, collagen deposits, and elevated serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (fig. S19, A, B, and E). Sirius red and cleaved Caspase-3 staining showed similar levels of collagen deposits and hepatocyte apoptosis in both genotypes (fig. S19, B to D). Serum levels of AST, ALT, and alkaline phosphatase (AP) also showed no significant differences (fig. S19E). Nonetheless, hepatocytes of Ang2-deficient mice showed ~2.5 times higher proliferation rates as compared with that of hepatocytes of WT mice (fig. S19, F and G).

LSEC-derived Ang2 is required to spatiotemporally orchestrate the proliferation of hepatocytes and LSEC to efficiently restore liver structure and function after hepatectomy (Fig. 4G): The rapid down-regulation of Ang2 after hepatectomy regulates hepatocyte proliferation in a paracrine (angiocrine) manner by down-regulating LSEC

TGFβ1 production, removing an endogenous growth inhibitory mechanism. During the angiogenic phase, recovery of Ang2 expression controls LSEC proliferation by regulating VEGFR2 expression in an autocrine manner. The dynamics of these divergent indirect and direct two-compartment effects are strictly controlled by the temporal regulation of LSEC Ang2 expression during liver regeneration (rapid down-regulation after hepatectomy and gradual recovery during the later angiogenic phase). Previous studies have shown that LSECs support liver regeneration in a stimulatory manner by secreting hepatotropic cytokines, including HGF and Wnt2 (3, 9). In this study, we establish that LSECs control liver regeneration in a much more dynamic manner through stimulatory and inhibitory effects. These data indicate a general mechanism by which signals transmitted from LSECs to hepatocyte play critical and rate-limiting roles in orchestrating liver regeneration. Collectively, the data shed fundamental insights into the role of the endothelium as a gatekeeper and regulator of tissue homeostasis and regeneration.

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Acknowledgments: We thank G. Thurston (Regeneron, Tarrytown, NY) for providing Ang2-deficient mice and critical discussions. We also thank A. Budnik and S. Savant (DKFZ Heidelberg, Germany) for important discussions and comments on the manuscript. We gratefully acknowledge the excellent technical support of F. Bestvater and M. Brom of the Microscopy Facility, M. Bewerunge-Hudler of the Microarray Unit, and the DKFZ laboratory animal core facility. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB-TR23 “Vascular Differentiation,” SFB-TR77 “HCC,” and SFB873 “Stem Cell Biology”), the DKFZ-MOST Cancer Research Cooperation, and the EU FP7 “SYSTEMAGE” (all to H.G.A.). H.G.A. is supported by an endowed chair from the Aventis Foundation. Microarray data were deposited in the Gene Expression Omnibus (GEO) database (accession no. GSE50046).

Supplementary Materials

www.sciencemag.org/content/343/6169/416/suppl/DC1
Materials and Methods
Figs. S1 to S20
Tables S1 to S3
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Movies S1 and S2

19 August 2013; accepted 12 December 2013
10.1126/science.1244880

Single β-Actin mRNA Detection in Neurons Reveals a Mechanism for Regulating Its Translatability

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The physical manifestation of learning and memory formation in the brain can be expressed by strengthening or weakening of synaptic connections through morphological changes. Local actin remodeling underlies some forms of plasticity and may be facilitated by local β-actin synthesis, but dynamic information is lacking. In this work, we use single-molecule *in situ* hybridization to demonstrate that dendritic β-actin messenger RNA (mRNA) and ribosomes are in a masked, neuron-specific form. Chemically induced long-term potentiation prompts transient mRNA unmasking, which depends on factors active during synaptic activity. Ribosomes and single β-actin mRNA motility increase after stimulation, indicative of release from complexes. Hence, the single-molecule assays we developed allow for the quantification of activity-induced unmasking and availability for active translation. Further, our work demonstrates that β-actin mRNA and ribosomes are in a masked state that is alleviated by stimulation.

More than a century ago, Ramón y Cajal postulated that memories could be stored by modifying the shape and, conse-

quently, the strength of synaptic connections between neurons (1). In our current understanding of learning and memory formation, synaptic plasticity

expressed as activity-induced changes in synapse morphology and, subsequently, in signaling strength constitutes one of the physical manifestations of memory formation (2). In postsynaptic forms of plasticity, activity-induced morphological remodeling and enhanced synaptic transmission can be specific to a single dendritic spine (3) and can require both protein synthesis (4) and increased polymerization of the cytoskeletal protein β -actin (5). Local protein synthesis provides a mecha-

nism of achieving spatial specificity of synaptic modification that can persist over time (6). β -actin mRNA localization in neuronal dendrites is essential for proper dendritic spine structure and abundance (7, 8), suggesting that regulation of β -actin protein concentration through local translation plays a role in synaptic plasticity.

Because β -actin mRNA is abundant in neurons (9), mRNAs must be maintained in a repressed state in the vicinity of synapses with translation factors readily available as needed for local translation. Physical sequestration of localized mRNAs in large neuronal RNA granule structures may serve to repress the mRNAs. RNA granules have been described as large, dense, neuronal-specific structures composed of ribosomes; mRNAs, including β -actin mRNA; and translation factors (10, 11). Putatively, local activity induces granule disassembly, spatially restricting translation to stimulated synapses (10, 12). To date, there exists

little evidence that granules regulate mRNA functionality. With the use of single-molecule imaging of endogenous β -actin mRNA and ribosomes, we provide evidence that the availability of β -actin mRNA to translation factors is transiently regulated by synaptic activity.

Single mRNA fluorescent in situ hybridization (FISH) analysis provided an absolute quantitation of dendritic mRNAs in cultured hippocampal neurons (13) (fig. S1). Probe hybridization efficiency to single molecules was calibrated with the brightness of a single probe (14). By this method, we found that dendritic mRNAs bind only half of the expected probes (Fig. 1, A and D, and fig. S2). However, exposure to chemical long-term potentiation (cLTP) induction, known to result in enlarged spines (15), increased probe binding to the expected number (11) and doubled detection of β -actin mRNAs along dendrites within 10 to 15 min (Fig. 1, B, D, and E; fig. S2, A and

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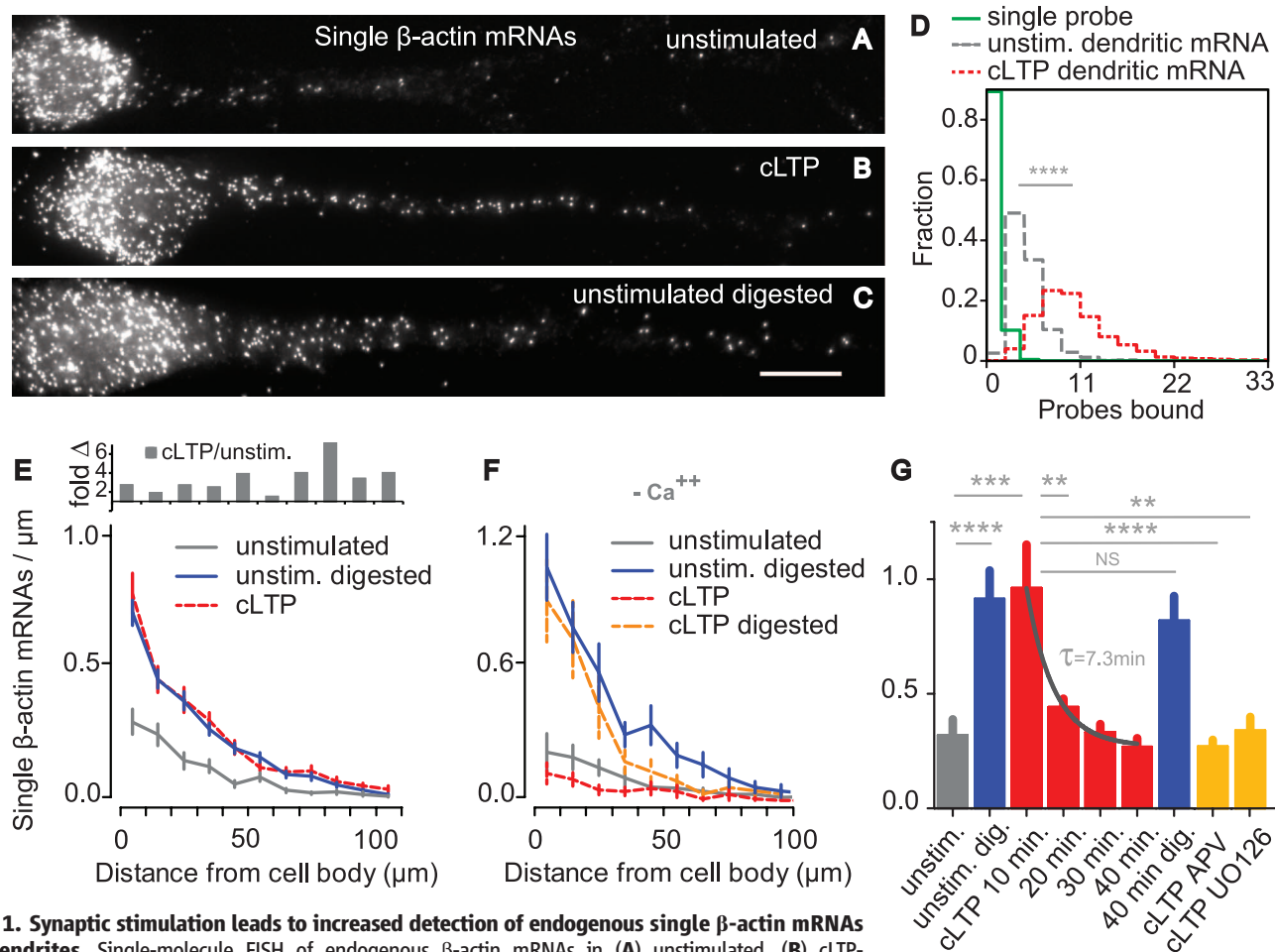


Fig. 1. Synaptic stimulation leads to increased detection of endogenous single β -actin mRNAs in dendrites. Single-molecule FISH of endogenous β -actin mRNAs in (A) unstimulated, (B) cLTP-stimulated, and (C) protease-digested cultured hippocampal neurons. Scale bar, 10 μ m. (D) Histogram of integrated intensities of single β -actin mRNAs in dendrites. After cLTP, single mRNAs bind the expected number of probes, although half without stimulation (unstim.) [**** $P < 0.0001$ (cLTP $n = 2728$ mRNAs, 25 cells; unstim. $n = 665$ mRNAs, 19 cells; single FISH probes $n = 2131$)]. (E) Single β -actin mRNAs per micrometer in dendrites. Fold increase of mRNA density along unstimulated to stimulated dendrites (top). From top to bottom in key: $n = 26$, 97, or 57 dendrites. (F) Single β -actin mRNAs per micrometer in dendrites with removal of extracellular calcium (from top to bottom: $n = 23$, 12, 19, or 7 dendrites). (G) Mean single mRNAs per micrometer in the first 50 μ m of dendrites. cLTP-induced unmasking of mRNA decays with a time constant (τ) of 7.3 min (fit: coefficient of determination $R^2 = 0.996$). Digestion 40 min after cLTP shows no decrease in mRNA, consistent with mRNA repackaging ($P = 0.5$). Increased mRNA detection is blocked by NMDA receptor inhibition (APV) or MEK1/2 inhibition (UO126) (** $P < 0.01$, **** $P = 0.001$, **** $P < 0.0001$; Student's t test). In order from left to right: $n = 26$, 21, 21, 35, 47, 44, 32, 31, or 23 dendrites. NS, not significant; dig., digested. Error bars in (E) to (G) denote SEM.

C; and fig. S3). Inefficient mRNA detection suggested inaccessibility of probes to dendritic mRNA, whereas stimulation induces unmasking. In contrast to neurons, β -actin mRNAs in glial cells in the same field hybridized the expected number of probes, irrespective of stimulation (fig. S2B). Probes to the β -actin 3' untranslated region (3' UTR), as well as the open reading frame, demonstrated mRNA unmasking (fig. S2, C and D). Increased mRNA was not due to transport from the soma or transcription (fig. S2, E to G).

If mRNA was masked by a proteinaceous complex, a limited prehybridization proteolytic digestion step would be expected to enhance mRNA detection. A protease digestion protocol was devised (16) that increased detectable β -actin mRNAs in dendrites equivalent to levels after stimulation (Fig. 1E). Poly(A) mRNA detection in dendrites also increased upon digestion (fig.

S2H), suggesting that additional mRNAs may be masked in neurons.

To be physiologically regulated, mRNA unmasking would require signaling cascades that occur during synaptic activity. Inhibition of *N*-methyl-D-aspartate (NMDA) receptor activity with 2-amino-5-phosphopentanoic acid (APV), inhibition of MEK1/2 (mitogen-activated protein kinase kinase 1 and 2) with UO-126, or depletion of calcium from the extracellular medium prevented increased mRNA detection during cLTP (Fig. 1, F and G), relating mRNA unmasking to local metabolic changes that occur during synaptic plasticity. Detectable dendritic β -actin mRNA peaked at 10 min after cLTP and returned to baseline within 30 min (Fig. 1G), indicating that the default condition is masking. Furthermore, limited mRNA availability for translation after cLTP could provide for a burst of protein synthesis.

Because repressive RNA granule complexes in neurons are composed of densely packed ribosomes (11), ribosomes may also be unmasked during cLTP. Similar to mRNA, cLTP increased endogenous ribosomal RNA (rRNA) detection along dendrites, as did sample digestion (Fig. 2A and fig. S4, A to E). Ribosomal particle intensities in protease-treated neuronal dendrites were nonhomogeneous and contained denser regions when compared with glia (fig. S4, F to H). Consistent with dispersal of ribosomes during unmasking, cLTP decreased the abundance of bright dendritic rRNA structures (fig. S4, E and I, and fig. S5F). To determine whether β -actin mRNA and ribosomes may be associated in the same particle as previously reported (11, 17), we performed dual-color hybridization in protease digested neurons (Fig. 2B). More than 30% of β -actin mRNAs colocalized with neuron-specific bright ribosomal structures.

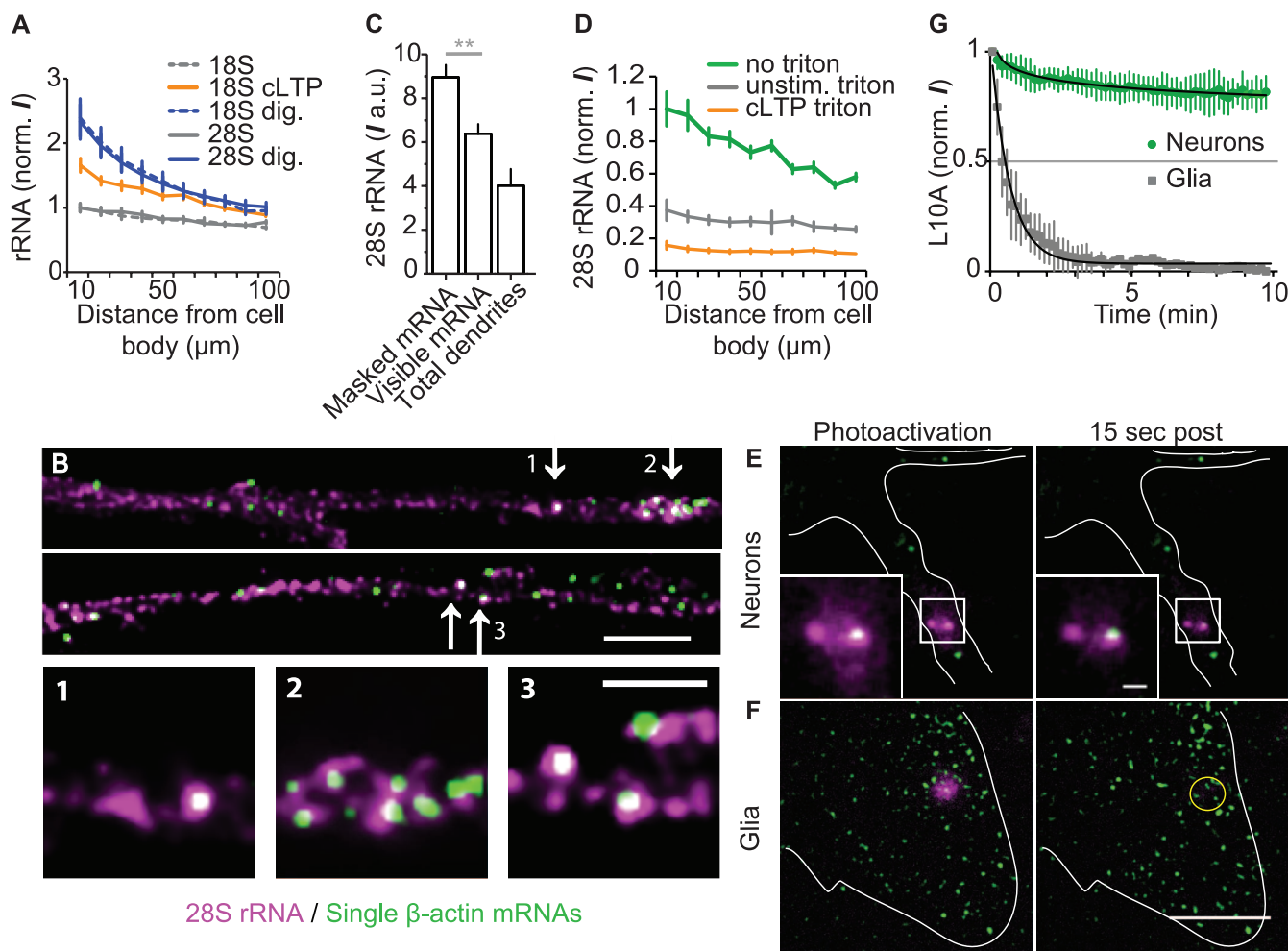


Fig. 2. Ribosomal complexes obscure β -actin mRNA. (A) Mean endogenous rRNA FISH fluorescence along dendrites (from top to bottom in key: $n = 49, 30, 52, 92$, or 97 dendrites). Signal normalized (norm.) to the first point of unstimulated data. Error bars denote SEM. I, intensity; dig., digested. (B) Single β -actin mRNAs and bright rRNA puncta often coincide (indicated by arrows). Scale bars, 5μ m (upper); 2μ m (lower). Numbers indicate magnified regions in bottom row. Magnified images were resampled with cubic convolution interpolation. (C) Mean dendritic ribosome signal surrounding mRNAs and total ribosomal signal ($n = 38$ dendrites, $**P < 0.01$; Student's *t* test). a.u., arbitrary units. (D) Mean dendritic rRNA FISH fluorescence in prefixative triton digested samples. Error bars indicate SEM. (E) Spot photoactivation of L10A-PA-TagRFPT at the site of β -actin mRNA in neurons revealed ribosome structures. Scale bar (inset), 1μ m. (F) In glia, L10A diffused away from the activated spot. Scale bar, 10μ m. (G) Loss of fluorescent L10A from photoactivated spot ($n = 6$ glia, 12 neurons). Error bars denote SD.

Three-color imaging differentiated ribosome association with masked and unmasked mRNAs (fig. S5A). β -actin mRNAs hybridized with a second-color probe after protease digestion revealed previously masked mRNAs (about half of the total). Masked mRNAs were associated with brighter ribosomal puncta, supporting the hypothesis that regions rich in ribosomes are part of the same masked complex (Fig. 2C and fig. S5, B to D).

Unbound and unmasked molecules should be washed away during permeabilization of live neurons. Puromycin eliminated actively translating polysomes, followed by detergent extraction and fixation (fig. S6A). Large, bright ribosomal puncta were retained in dendrites, some containing single β -actin mRNAs; in contrast, glial cytoplasmic ribosomal signal was extracted (fig. S6, B to D and H). After cLTP in detergent-treated neurons, ribosome and β -actin mRNA FISH signal (Fig. 2D and fig. S6E) and the size of the optically reconstructed ribosomal puncta (fig. S6, F and G) decreased by about half, consistent with increased dispersal of ribosomes and mRNAs as a result of synaptic stimulation.

Increased ribosomal dispersal due to unmasking resulted in increased diffusion of ribosomes and mRNAs. To investigate dendritic rRNA dynamics, we employed spot photoactivation of a ribosomal protein (L10A) fusion. Immobile, bright structures surrounded single mRNAs (Fig. 2E). Photoactivated ribosome fusions exhibited diminished motility in dendrites relative to glial cells (Fig. 2G). After cLTP, quantification of ribosome dispersal from photoactivated sites revealed that the immobile ribosome fraction decreased (fig. S7B). Dendritic β -actin mRNAs were immobile, with a small portion undergoing active transport (18). Upon stimulation, immobile mRNAs exhibited corralled diffu-

sion in their local environment (fig. S7, C and D), consistent with degranulation of a complex that prevents mRNA from diffusing until synaptic stimulation.

In addition to increased mRNA and ribosome dynamics, cLTP increased protein synthesis of a reporter for β -actin mRNA, consistent with unmasking that correlates with increased local β -actin translation (fig. S8). The protein FMRP has been shown to be a component of RNA granules and stalls ribosomes on mRNAs (19). *Fmr1* knockout brains exhibit a reduction in the postpolysomal fractions (20), suggesting a role for *Fmr1* in granule integrity. Accordingly, *Fmr1* knockdown in culture decreased the abundance of masked β -actin mRNAs (fig. S9, A to C). We observed a similar effect in neurons isolated from knockout animals lacking the β -actin mRNA binding protein ZBP1 (fig. S9D).

In the model supported by this work, β -actin mRNA is present all along dendrites and is kept in a dormant state by packaging into inert structures ready to be locally activated. During synaptic stimulation, downstream effectors of signaling pathways locally prompt complex disassembly, putatively allowing active translation of mRNAs at activated synapses. This could be accomplished by regulation of self-aggregating protein motifs through posttranslational modifications (21) facilitating cycles of localized RNA granule formation and degranulation, thus making use of the same mRNAs repeatedly over time.

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Acknowledgments: We thank G. J. Bassell for the gift of the Dendra-actin 3'UTR construct, J. Du Hoffmann for invaluable programming help, D. R. Larson for Localize software, and Y. J. Yoon for cloning L10A and helpful discussions. We also thank T. Trček and other past and present members of the Singer lab for their suggestions and support. This work was supported by NIH grant NS083085-19 (formerly GM84364) and the Weisman Family Foundation. Author notes: A.R.B., B.W., and R.H.S. designed the experiments; A.R.B. performed the experiments; A.R.B. and B.W. performed optical engineering and data analysis; and A.R.B. and R.H.S. wrote the manuscript. Data in this paper are in partial fulfillment of the Ph.D. degree to A.R.B.

Supplementary Materials

www.sciencemag.org/content/343/6169/419/suppl/DC1

Materials and Methods

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FISH Probe Sequences

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9 July 2013; accepted 4 December 2013

10.1126/science.1242939

Visualization of Dynamics of Single Endogenous mRNA Labeled in Live Mouse

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The transcription and transport of messenger RNA (mRNA) are critical steps in regulating the spatial and temporal components of gene expression, but it has not been possible to observe the dynamics of endogenous mRNA in primary mammalian tissues. We have developed a transgenic mouse in which all β -actin mRNA is fluorescently labeled. We found that β -actin mRNA in primary fibroblasts localizes predominantly by diffusion and trapping as single mRNAs. In cultured neurons and acute brain slices, we found that multiple β -actin mRNAs can assemble together, travel by active transport, and disassemble upon depolarization by potassium chloride. Imaging of brain slices revealed immediate early induction of β -actin transcription after depolarization. Studying endogenous mRNA in live mouse tissues provides insight into its dynamic regulation within the context of the cellular and tissue microenvironment.

Recent advances have provided insights into the behavior of RNA in real time (1). However, most live-cell imaging techniques require transfection or injection of ex-

ogenous reporters that are typically overexpressed or are missing regulatory elements and binding partners present in the endogenous molecules. Moreover, immortalized cells may not accurately

exhibit RNA regulation representative of the native tissue environment.

To address these limitations, we generated a transgenic mouse in which all endogenous β -actin mRNA is fluorescently labeled by specific binding between the MS2 bacteriophage capsid protein (MCP) and the MS2 binding site (MBS) RNA stem-loops (2). Lentiviral transgenesis (3) was used to integrate the MCP-GFP (green fluorescent

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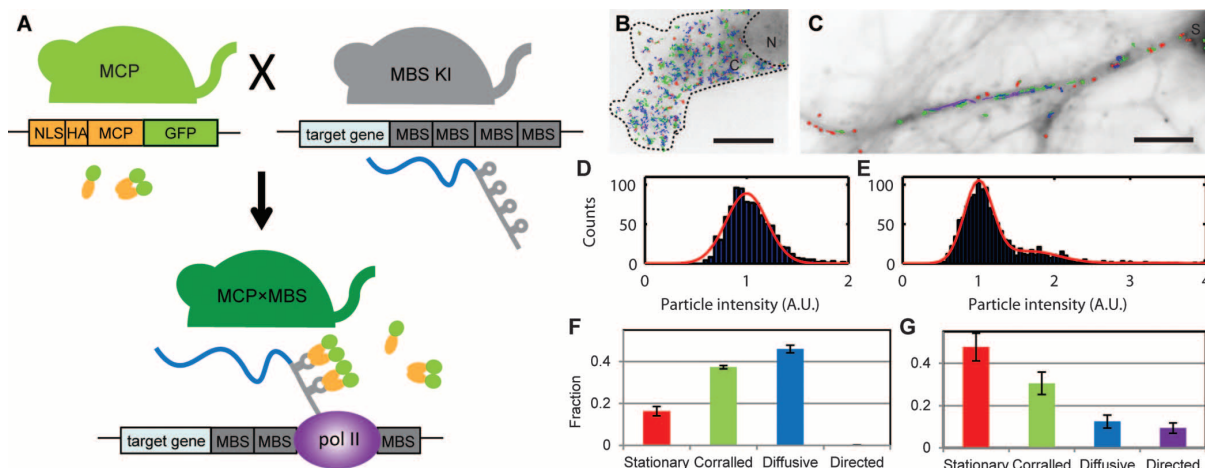


Fig. 1. Labeled endogenous mRNA in MCPxMBS mouse. (A) Schematic for in vivo RNA labeling. NLS, nuclear localization sequence; HA, hemagglutinin; pol II, RNA polymerase II. (B and C) Single-particle tracking of GFP-labeled β -actin mRNA in primary MEF (B) and hippocampal neuron (C) from mouse. Track classifications: red, stationary; green, corralled; blue, diffusive; purple, directed motion

(see supplementary text). N, nucleus; C, cytoplasm; S, soma. Scale bars, 10 μ m. (D and E) Intensity histograms of β -actin mRNPs tracked in primary MEFs (D) and neurons (E). Red curves show one- and three-component Gaussian fits for (D) and (E), respectively. (F and G) Fraction of stationary, corralled, diffusive, and directed β -actin mRNPs in primary MEFs (F) and neurons (G). Error bars denote SEM ($n = 6$ cells).

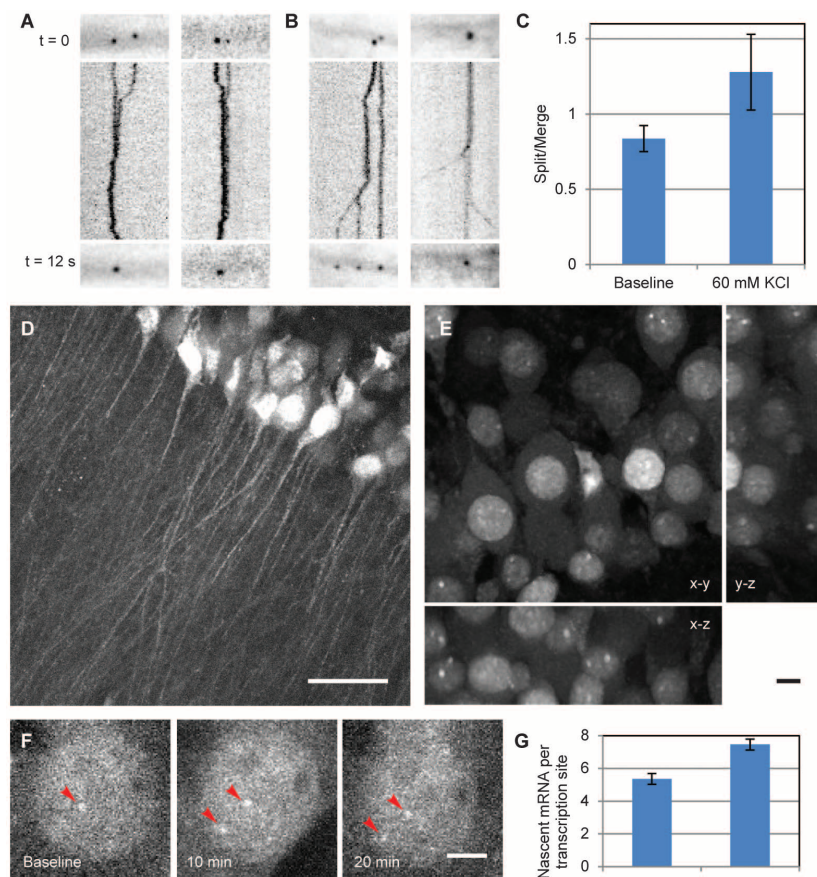


Fig. 2. Activity-dependent dynamics of β -actin mRNA in neurons from MCPxMBS mouse. (A and B) Examples of β -actin mRNA merge (A) and split (B) events. From top to bottom: initial images of the particles, kymographs during a 12-s period, and final images. (C) Ratio of split to merge events increased after KCl depolarization ($n = 11$ neurons from three cultures, 14 to 16 days in vitro; $P < 0.05$ by t test). (D) CA1 region in acute hippocampal slice. Scale bar, 50 μ m. (E) Soma layer of CA1 neurons. Panels show maximum projections of x-y, y-z, and x-z planes. Scale bar, 10 μ m. (F) Transcription of β -actin gene with KCl depolarization. Arrowheads denote β -actin transcription sites. Scale bar, 5 μ m. (G) Nascent mRNA per transcription site increased after depolarization ($n = 86$ sites, brain slices from 3 mice; $P < 0.001$ by pairwise t test). Error bars denote SEM.

protein) transgene with high efficiency (fig. S1). The resulting MCP mice were crossed with Actb-MBS mice, in which 24 repeats of MBS are knocked into the 3' untranslated region (UTR) of the β -actin gene (4), to label endogenous β -actin mRNA with GFP, resulting in MCPxMBS mice (Fig. 1A). β -Actin mRNA, which is essential for early embryonic development (5), was labeled with the 1200-nucleotide MBS cassette and up to 48 molecules of MCP-GFP (fig. S2A), yet no abnormalities were found by histologic analysis ($n = 3$ mice); mice were fertile (fig. S2B), and β -actin mRNA and protein expression levels were similar to those of wild-type mice (fig. S2, C and D). Hence, MS2-GFP labeling of endogenous β -actin mRNA did not disrupt its function and the expression level in vivo, confirming the physiological relevance of the studies.

Localization of β -actin mRNA was observed in primary fibroblast leading edges (6), neuronal growth cones (7), and mature neuron dendrites and spines (8) by fluorescence in situ hybridization (FISH). However, it is unclear how β -actin mRNA localizes in real time. Because loss of mRNA localization occurs in culture, we imaged mouse embryonic fibroblasts (MEFs) from MCPxMBS mice within 48 hours after isolation (Fig. 1B, fig. S3, and movie S1). Individual mRNA-protein complex (mRNP) particles were identifiable in MCPxMBS cells, unlike the background in MCP cells (fig. S2, E and F). Both single-molecule FISH and GFP labeling in live cells showed that these particles contained only single copies of β -actin mRNA (Fig. 1D, fig. S4, and supplementary text). The ensemble diffusion coefficient of the labeled endogenous β -actin mRNA was $0.09 \pm 0.02 \mu\text{m}^2/\text{s}$, similar to a reporter mRNA in a cell line (9). However, the movement patterns of endogenous mRNA appeared different from those of exogenous mRNA. There was less directed

motion (~1%) of endogenous mRNA (Fig. 1F and fig. S5) than previously reported (22%) (9), possibly due to the differences between the endogenous mRNA and the exogenous reporter, or the cell types. Serum-induced localization of β -actin mRNA in fibroblasts appears predominantly mediated by rapid release of stationary mRNA and redistribution into discrete cytoplasmic compartments (fig. S6, movies S2 to S5, and supplementary text), although we cannot rule out short movements driven by nonprocessive motors.

Neuronal RNA transport granules may contain multiple mRNAs (10, 11). To investigate the stoichiometry of β -actin mRNA in hippocampal neurons from MCP×MBS mice, we performed single-molecule FISH (fig. S7). The intensity histograms of diffraction-limited fluorescent spots indicated mRNPs containing multiple copies of β -actin mRNA in the soma and proximal dendrites (fig. S7B), which decreased with distance from the soma (fig. S7D). In live neurons (Fig. 1C and movie S6), ~25% of mRNPs in proximal dendrites contained more than one β -actin mRNA (Fig. 1E). Diffusion of mRNPs in neurons was slower [diffusion coefficient = $3.8 (\pm 0.5) \times 10^{-3} \mu\text{m}^2/\text{s}$] than in fibroblasts, but ~10% of mRNPs were actively transported anterograde and retrograde (Fig. 1G and fig. S8A) with a mean speed of $1.3 \mu\text{m/s}$ (fig. S8B). The ratio of anterograde to retrograde transport was 1.1 to 1.5 throughout neuronal development in culture (fig. S8C), which may mediate constitutive delivery into dendrites.

To investigate the activity-dependent dynamics of β -actin mRNA, we imaged live neurons before and after depolarization (60 mM KCl for 3 to 6 min). Pairwise comparisons in the same dendritic regions revealed that there were significant increases in the density of the mRNP particles after KCl depolarization in both cultured

neurons (fig. S9) (8) and acute brain slices (fig. S10). The diffusion coefficient decreased by a factor of 3 (fig. S9D), and particles with directed motion decreased in both directions (fig. S9E). Therefore, the increase of diffraction-limited spots in the dendrite was not due to transport of mRNA from the soma. We hypothesized that the number of detected spots increased because of the release of mRNAs from mRNP complexes upon stimulation. We quantified the number of β -actin mRNAs contained in each neuronal mRNP by particle intensity. After KCl depolarization, the number of spots containing single β -actin mRNA increased while the number of particles bearing multiple mRNAs decreased (fig. S9B and S10B). We observed merge and split events of particles (Fig. 2, A and B, and movies S7 and S8). Both the split and merge frequencies were reduced after depolarization, but the ratio of split to merge was increased (Fig. 2C). These results suggest that mRNA molecules undergo continuous assembly and disassembly of large mRNP complexes (12) but favor the released state upon depolarization, possibly for local translation (13, 14).

We investigated endogenous β -actin gene expression in native tissue by imaging acute brain slices (Fig. 2D). Transcriptional activity was monitored in the hippocampus CA1 region at 20 to 60 μm from the surface before and after KCl depolarization (Fig. 2E). Nascent β -actin mRNA per transcription site increased 10 to 15 min after depolarization (Fig. 2, F and G), likely because of rapid initiation (15). Rapid induction of β -actin transcription was observed in various cell lines (4, 16, 17) but β -actin was not recognized as an immediate early gene in the nervous system, probably because of high basal expression. Increased expression of β -actin may be implicated in transducing synaptic activity into structural plasticity.

The MCP×MBS mouse provides a distinctive tool for monitoring the dynamics of single endogenous mRNA in live mammalian cells and tissues. Our results with the β -actin gene suggest that the technique could be generally applicable to other genes to investigate the effect of the tissue microenvironment on single-cell gene expression.

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Acknowledgments: Supported by NIH grants EB13571 and NS083085-19 (formerly GM84364) (R.H.S.) and National Research Service Award F32-GM87122 and the Integrated Imaging Program (H.Y.P.).

Supplementary Materials

www.sciencemag.org/content/343/6169/422/suppl/DC1
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16 April 2013; accepted 15 October 2013
10.1126/science.1239200

The HydG Enzyme Generates an Fe(CO)₂(CN) Synthon in Assembly of the FeFe Hydrogenase H-Cluster

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Three iron-sulfur proteins—HydE, HydF, and HydG—play a key role in the synthesis of the [2Fe]_H component of the catalytic H-cluster of FeFe hydrogenase. The radical *S*-adenosyl-L-methionine enzyme HydG lyses free tyrosine to produce *p*-cresol and the CO and CN[−] ligands of the [2Fe]_H cluster. Here, we applied stopped-flow Fourier transform infrared and electron-nuclear double resonance spectroscopies to probe the formation of HydG-bound Fe-containing species bearing CO and CN[−] ligands with spectroscopic signatures that evolve on the 1- to 1000-second time scale. Through study of the ¹³C, ¹⁵N, and ⁵⁷Fe isotopologs of these intermediates and products, we identify the final HydG-bound species as an organometallic Fe(CO)₂(CN) synthon that is ultimately transferred to apohydrogenase to form the [2Fe]_H component of the H-cluster.

FeFe hydrogenase enzymes rapidly evolve H₂ at a 6-Fe catalytic site termed the H-cluster (Fig. 1A) (1–3), which comprises

a traditional 4Fe-4S subcluster ([4Fe-4S]_H), produced by canonical Fe-S cluster biosynthesis proteins, that is linked via a cysteine bridge to

a dinuclear Fe subcluster ([2Fe]_H) that contains unusual ligands. Specifically, the [2Fe]_H subcluster possesses two terminal CN[−] ligands, two terminal CO ligands, and azadithiolate and CO bridges, all of which are thought to be synthesized and installed by a set of Fe-S proteins denoted HydE, HydF, and HydG. In one recent model for the [2Fe]_H subcluster bioassembly pathway (4, 5), the two radical *S*-adenosyl-L-methionine (SAM) enzymes of the set, HydE and HydG, generate the dithiolate moiety and free CO and CN[−], respectively, and these ligands are then transferred to a dinuclear Fe precursor bound to HydF,

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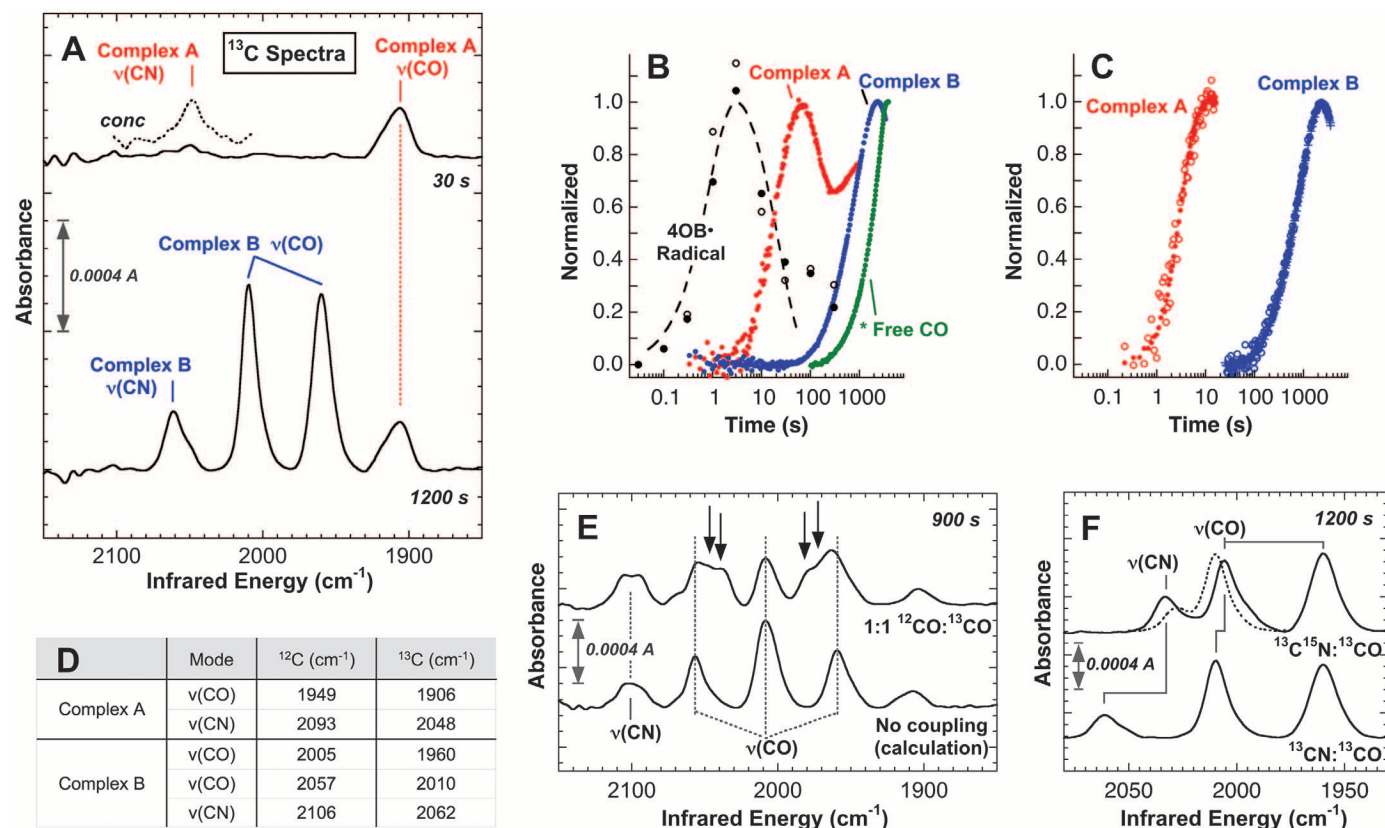
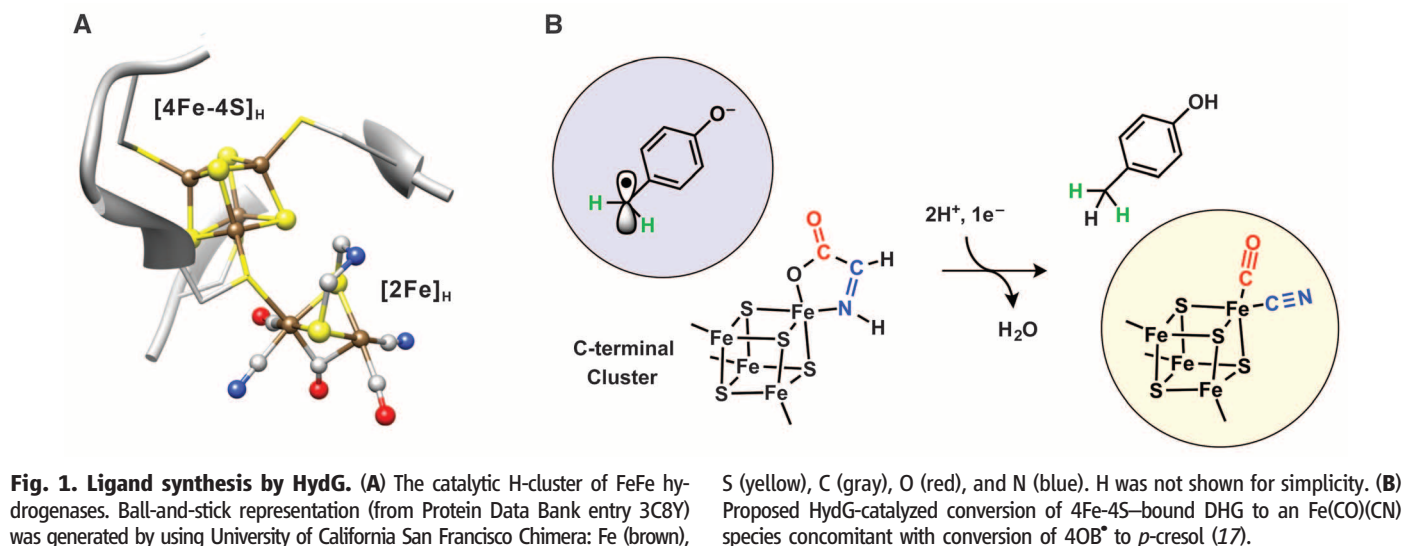
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which acts as a scaffold protein for assembling the final $[2\text{Fe}]_{\text{H}}$ subunit. Individually expressed HydE, HydF, and HydG can be combined for

successful *in vitro* synthesis of the $[2\text{Fe}]_{\text{H}}$ component of the H-cluster and concurrent activation of FeFe hydrogenase apoprotein (6, 7). Alter-

natively, an abiotically synthesized dinuclear Fe subcluster, constructed with an azadithiolate bridge as well as one CN^- and two CO ligands per Fe,



•, $\nu(^{13}\text{CO})$ 2010 cm^{-1} ; +, $\nu(^{13}\text{CO})$ 1960 cm^{-1} . The data for complex A were taken from measurements by using $800\ \mu\text{M}$ HydG^{WT} in order to enhance the signal:noise ratio of the $\nu(\text{CN})$ mode. (D) Table of frequencies for observed IR bands of complexes A and B prepared by using Tyr (middle column) or $(^{13}\text{C})_9\text{-Tyr}$ (right-most column). (E) SF-FTIR spectrum of complex B measured at 900 s and prepared by using a 1:1 mixture of Tyr and ^{13}CO -Tyr (top). Average of the ^{12}CO and ^{13}CO product B spectra (bottom). The arrows indicate new bands not present in either the ^{12}CO or ^{13}CO spectra of complex B. (F) SF-FTIR spectra of complex B measured at 1200 s and prepared by using $(^{13}\text{C})_9\text{-}^{15}\text{N-Tyr}$ (top) and $(^{13}\text{C})_9\text{-Tyr}$ (bottom). Expected CN and CO bands for $^{13}\text{C}^{15}\text{N}$ -containing complex B in the absence of $\nu(\text{CN})/\nu(\text{CO})$ vibrational mixing (dotted line). Predicted band shifts computed simply by the change in the reduced mass.

can also be used to activate FeFe hydrogenase (8, 9).

The HydG radical SAM enzyme was previously reported to use free L-tyrosine (Tyr) as its substrate to generate *p*-cresol and free CO and CN[−] as its products (6, 10–12). Although HydG has yet to be crystallographically characterized, sequence analysis and results from spectroscopic studies indicate that it has two 4Fe-4S clusters, each with distinct functions (13, 14). The SAM-binding 4Fe-4S cluster, located near the N terminus, reductively cleaves SAM, producing methionine plus a strongly oxidizing 5'-deoxyadenosyl radical (5'-dA[•]) (15, 16). The second Fe-S cluster, located near the C terminus, was implicated as the site of Tyr binding in our recent investigation of the HydG reaction mechanism using electron paramagnetic resonance (EPR) spectroscopy (17). Also on the basis of EPR spectroscopic results, we proposed that the initial 5'-dA[•] generates a neutral tyrosine radical bound to this C-terminal 4Fe-4S cluster, which then undergoes heterolytic cleavage at the C_α-C_β bond, forming a 4-oxidobenzyl radical (4OB[•]) and cluster-bound dehydroglycine (DHG) (Fig. 1B) (17). Electron and proton transfer to 4OB[•] then yields the *p*-cresol product concomitant with the scission of DHG to form Fe-bound CO and CN[−] and water (Fig. 1B). In this report, we

explored the subsequent time course of the HydG reaction by using stopped-flow Fourier transform infrared (SF-FTIR) and electron-nuclear double resonance (ENDOR) spectroscopies to follow the synthesis of Fe(CO)₂(CN)₂ species and track them to the completion of the H-cluster bioassembly pathway.

SF-FTIR is a useful time-resolved method for studying the HydG reaction mechanism because CO and CN[−] ligands give rise to strong infrared absorption bands with energies and intensities sensitive to the coordination and electronic environment of the bound Fe center. Figure 2 summarizes SF-FTIR data after the reaction of wild-type *Shewanella oneidensis* HydG (HydG^{WT}) with excess substrates SAM and ¹³C-labeled Tyr [(¹³C)₉-Tyr] in the presence of the reductant sodium dithionite (DTH). Observed bands are assigned to stretch vibrations of CO and CN ligands on the basis of their energy shifts upon ¹²C:¹³C and ¹⁴N:¹⁵N site-specific isotope substitution (fig. S2), which was achieved through use of the appropriate Tyr isotopologs (6). The time evolution of the resultant spectra (Fig. 2, A to C) reveals a stepwise conversion of a discrete intermediate, which we term complex A, to a distinct new species, complex B. Complex A is characterized by two bands at 1906 cm^{−1} and 2048 cm^{−1} [1949 cm^{−1} and 2093 cm^{−1} with ¹²C-Tyr

(Fig. 2D)], which we assign to stretching modes arising from terminal Fe-¹³CO and Fe-¹³CN moieties, respectively. The kinetic profile (Fig. 2B) shows that complex A accumulates on the same time scale as the decay of the previously observed 4OB[•] (17). Hence, the FTIR spectrum and formation kinetics of complex A are consistent with the mechanistic model in Fig. 1B, with the first turnover of HydG, SAM, and Tyr forming bound CO and CN[−], presumably on the unique Fe site of the C-terminal 4Fe-4S cluster. Complex A does not form in the relatively conservative Cys³⁹⁴→Ser³⁹⁴ (C394S), C397S HydG double mutant (HydG^{SxxS}), which lacks the C-terminal cluster (figs. S3 and S4).

Complex A reaches a maximum concentration and starts to decay after about 30 s, concomitant with the appearance and growth of complex B. The three bands associated with complex B have identical kinetics and may be assigned to terminally bound ligands: two high-energy, predominantly ν(¹³CO) modes at 1960 cm^{−1} and 2010 cm^{−1} and a ν(¹³CN) mode at 2062 cm^{−1}. We assign the 2010 cm^{−1} band to a predominantly ν(¹³CO) mode rather than a ν(¹³CN) mode because its energy shifts upon ¹³C isotopic substitution at the carboxyl position in Tyr [the moiety that gives rise to the CO but not the CN ligands in the mature H cluster (fig. S2)] (6). An additional band at 1906 cm^{−1} and shoulder at 2048 cm^{−1} likely arise from residual complex A.

A reasonable structure for complex B is a cuboidal Fe(CO)₂(CN)-[3Fe-4S] species in which the unique Fe site is coordinated by the three Tyr-derived diatomic ligands. For CO and CN[−] ligands that are bound to a single Fe center, the ν(CO) and ν(CN) vibrational modes are expected to be strongly coupled. To test for vibrational mixing between the two observed ν(CO) frequencies, we generated a 1:2:1 mixture of ¹²CO¹²CO-, ¹²CO¹³CO-, and ¹³CO¹³CO-labeled complex B from a 1:1 mixture of natural abundance Tyr and ¹³COO-Tyr. If the two ν(CO) modes in complex B were not coupled, then the expected resulting spectrum would be a simple 1:1 superposition of the ¹²CO¹²CO and ¹³CO¹³CO spectra (Fig. 2E, lower trace). Instead, the observed spectrum displayed additional, overlapping bands (Fig. 2E, upper trace) that can be ascribed to the two ¹²CO¹³CO-labeled isotopomers with apparent CO-stretching frequencies shifted relative to their

Fig. 3. Q-band (34.00 GHz, 1.155 T) Davies ENDOR spectra of FeFe hydrogenase HydA1 enriched with ⁵⁷Fe. (A) HydA1⁵⁷Fe; (B) HydA1⁵⁷Fe-HydG [black, experiment; red, simulation with A(⁵⁷Fe) = 16.0 MHz]; (C) (A) − (B) difference spectrum [black, experiment; red, simulation with A(⁵⁷Fe) = 10.55 MHz]. ENDOR simulations used line widths of 0.4 and 0.65 MHz for [2Fe]_H and [4Fe-4S]_H components, respectively, with other values taken directly from experiment parameters and CW EPR simulations. The ENDOR spectrum of natural abundance Fe HydA1 in the H_{ox} state was subtracted from both spectra (A) and (B). a.u., arbitrary units.

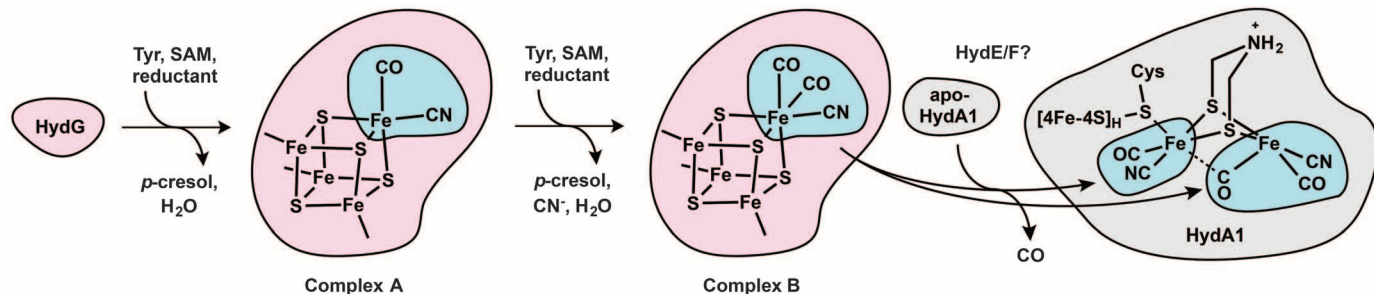
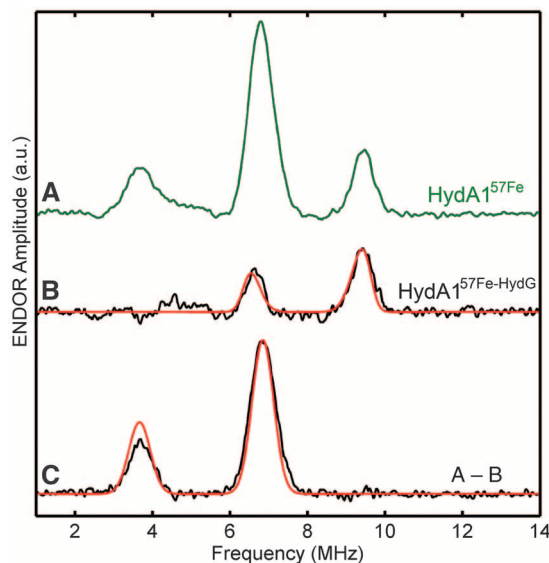


Fig. 4. Schematic for the bioassembly of the FeFe hydrogenase H-cluster via a HydG-synthesized Fe(CO)₂(CN)₂ synthon. Pink indicates HydG; cyan, the unique Fe site of HydG that is installed into apo HydA1; gray, HydA1.

isotopically pure counterparts because of vibrational mixing of the two fundamental $\nu(\text{CO})$ modes (fig. S6). Similarly, a comparison of spectra generated by using $(^{13}\text{C})_9\text{-Tyr}$ and $(^{13}\text{C})_9\text{-}^{15}\text{N-Tyr}$ (Fig. 2F) shows that ^{15}N substitution produces both a smaller than expected energy shift in the $\nu(\text{CN})$ mode as well as an energy shift in the higher energy $\nu(\text{CO})$ mode, indicating that these modes are also vibrationally mixed. Taken together, these coupling data suggest that all three ligands (two CO and one CN^-) in complex B are bound to a single Fe-center.

The relatively high energies of the $\nu(\text{CO})$ modes of complex B suggest that it may be appropriately modeled as a cationic, low-spin $[\text{Fe}(\text{II})(\text{CO})_2(\text{CN})]^+$ complex based on comparisons with related neutral, low-spin $\text{Fe}(\text{II})(\text{CO})_2(\text{CN})$ complexes (18, 19). The density functional theory (DFT)-calculated $\nu(\text{CO})$ and $\nu(\text{CN})$ frequencies and intensities of $[\text{fac}-(\text{H}_2\text{S})_3\text{Fe}(\text{II})(\text{CO})_2(\text{CN})]^+$, a simple model for $\{\text{Fe}(\text{II})(\text{CO})_2(\text{CN})\text{-}[3\text{Fe-4S}]\}^+$, show reasonable agreement with the observed spectrum of complex B (fig. S5 and table S2). Alternative structures in which the CO and CN^- ligands are not cofacial or in which the sulfur donors are replaced with oxygen donors were not found to give rise to the expected intensity patterns. In addition, the coupling pattern in the $^{12}\text{C}/^{13}\text{C}$ isotope mixture (Fig. 2E, top) was only reproduced computationally when the two CO ligands have different inherent $\nu(\text{CO})$ energies, which could be explained by the electronic asymmetry of the coordinating $[3\text{Fe-4S}]^0$ subcluster (20) or by asymmetry in CO H-bonding interactions.

The kinetics in Fig. 2B suggest sequential conversion of complex A to complex B, which we associate with another turnover of HydG acting on a second substrate Tyr to form an additional CO to coordinate the unique Fe center of the C-terminal cluster (21). Unlike the first turnover (17), we have no experimental information at this time about how Tyr binds and what intermediates may be involved in this second reaction, although we note that our model for complex A is coordinatively unsaturated and could bind Tyr or a Tyr-derived fragment during the second turnover. Only at much longer times was free CO detected in solution through its binding to exogenous myoglobin (see green trace in Fig. 2B and fig. S3), which rules out the binding of externally derived CO as part of the mechanism. The release of free CO is correlated with the rise of complex A (Fig. 2B) at a late time (>300 s), suggesting that complex B may degrade to complex A by loss of CO when other H-cluster maturation components are not available to facilitate its incorporation into the proper downstream assembly products (vide infra). Reports of no lag phase in free CO production (12) may arise from alternative chemistry resulting from differences in experimental protocols.

To determine the fate of the Fe-containing complex B, we measured the continuous-wave (CW) EPR and pulse ENDOR spectra of HydA1 hydrogenase from *Chlamydomonas reinhardtii*

that had been either uniformly or selectively labeled with ^{57}Fe (nuclear spin, $I = 1/2$). Uniformly ^{57}Fe -labeled HydA1 (HydA1- ^{57}Fe) was generated by introducing ^{57}Fe into the growth media for HydA1. The Q-band Davies pulse ENDOR (22) spectrum of the purified HydA1- ^{57}Fe poised in the H_{ox} state is shown in Fig. 3A. This spectrum was obtained at the highest electron g value, $g_1 = 2.10$, where there is no overlap with the EPR signal from the CO-inhibited $\text{H}_{\text{ox}}\text{-CO}$ form (fig. S7), and it shows several peaks in the 3- to 10-MHz range.

In order to determine whether Fe from the HydG cluster is transferred to the HydA1 hydrogenase, we evaluated a sample of HydA1 (that we term HydA1- $^{57}\text{Fe-HydG}$) in which the $[\text{2Fe}]_{\text{H}}$ subcluster was assembled in vitro (6, 7) by using HydE and HydF expressed in natural abundance media and HydG expressed in ^{57}Fe -enriched media (fig. S1). The corresponding ENDOR spectrum (Fig. 3B) was well simulated by a single doublet, with frequencies $\nu_{\pm} = A(^{57}\text{Fe})/2 \pm \nu_1$, where $A(^{57}\text{Fe}) = 16.0$ MHz is the effective hyperfine tensor component at this $g_1 = 2.10$ value and ν_1 is the ^{57}Fe nuclear Zeeman frequency at this magnetic field. We assigned this doublet to the $[\text{2Fe}]_{\text{H}}$ subcluster, because the $[\text{4Fe-4S}]_{\text{H}}$ subcluster is not labeled with ^{57}Fe for this sample. Subtraction of the ^{57}Fe ENDOR spectrum of the $[\text{2Fe}]_{\text{H}}$ subcluster-labeled sample from that of the HydA1- ^{57}Fe sample isolated the ^{57}Fe ENDOR signal that arose from the $[\text{4Fe-4S}]_{\text{H}}$ cluster (Fig. 3C). This difference spectrum was well simulated as an ENDOR doublet with $A(^{57}\text{Fe}) = 10.55$ MHz. Both $A(^{57}\text{Fe})$ values for HydA1 are consistent with previous ENDOR and Mössbauer spectroscopic studies of the H_{ox} state of *Desulfovibrio vulgaris* and *Clostridium pasteurianum* hydrogenases (≈ 16 to 18 MHz for the $[\text{2Fe}]_{\text{H}}$ subcluster and ≈ 8 to 10 MHz for the $[\text{4Fe-4S}]_{\text{H}}$ cluster) (23–26), although they differ somewhat from those derived from ENDOR data for the *Desulfovibrio desulfuricans* hydrogenase (12.4 and 11.1 MHz, respectively) (27). Comparing the magnitudes of the two $A(^{57}\text{Fe})$ values determined above confirms that the greatest spin density of the H-cluster in the H_{ox} state lies on the $[\text{2Fe}]_{\text{H}}$ subcluster. More importantly, these ENDOR data show that Fe in the $[\text{2Fe}]_{\text{H}}$ subcluster of the mature H-cluster originates from the HydG radical SAM maturase.

The SF-FTIR and ^{57}Fe ENDOR spectroscopic results presented in this report provide further insight into the assembly of the $[\text{2Fe}]_{\text{H}}$ subcluster of FeFe hydrogenase, illustrated in Fig. 4. Prior work demonstrated that the CO and CN^- ligands of the $[\text{2Fe}]_{\text{H}}$ subcluster are generated from Tyr via HydG. Results from SF-FTIR spectroscopic studies provide evidence for the formation of two distinct Fe-bound CO- and CN-containing species, complexes A and B, during the HydG reaction. Analysis of the vibrational mode coupling, buttressed by DFT studies on model complexes, lead us to postulate that complex B is a 3Fe-4S -bound $\text{Fe}(\text{CO})_2(\text{CN})$ species. The ^{57}Fe -labeling experiments prove that Fe in the $[\text{2Fe}]_{\text{H}}$ subcluster is provided by HydG. It is therefore

straightforward to envisage a mechanism wherein two complex B units assemble together with a dithiolate bridge to form the $[\text{2Fe}]_{\text{H}}$ subcluster. Our proposal for the structure of this deliverable species is consistent with all of the available data and points to the biologically relevant product of the HydG reaction as being an organometallic $\text{Fe}(\text{CO})_2(\text{CN})$ synthon.

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- This process would also entail formal loss of CN^- . Although the SF-FTIR data for complex B cannot rigorously exclude coordination by two CN^- groups, we consider this to be unlikely because only one $\nu(\text{CN})$ mode is observed and only one CN^- per Fe is present in both the assembled $[\text{2Fe}]_{\text{H}}$ subcluster and the synthetic dinuclear Fe compound that can activate the FeFe hydrogenase (8, 9).
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Acknowledgments: This work was funded by the NIH (R.D.B., no. GM072623; S.P.C., no. GM65440) and by the Division of Material Sciences and Engineering (J.R.S., award no. DE-FG02-09ER46632) of the Office of Basic Energy Sciences of the U.S. Department of Energy (DOE), by the Office of Biological and Environmental Research of the DOE (S.P.C.), and the UniCat Cluster of Excellence of the German Research Council (V.P.).

Supplementary Materials

www.sciencemag.org/content/343/6169/424/suppl/DC1
Materials and Methods
Figs. S1 to S10
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30 September 2013; accepted 9 December 2013
10.1126/science.1246572

IFI16 DNA Sensor Is Required for Death of Lymphoid CD4 T Cells Abortively Infected with HIV

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The progressive depletion of quiescent “bystander” CD4 T cells, which are nonpermissive to HIV infection, is a principal driver of the acquired immunodeficiency syndrome (AIDS). These cells undergo abortive infection characterized by the cytosolic accumulation of incomplete HIV reverse transcripts. These viral DNAs are sensed by an unidentified host sensor that triggers an innate immune response, leading to caspase-1 activation and pyroptosis. Using unbiased proteomic and targeted biochemical approaches, as well as two independent methods of lentiviral short hairpin RNA-mediated gene knockdown in primary CD4 T cells, we identify interferon- γ -inducible protein 16 (IFI16) as a host DNA sensor required for CD4 T cell death due to abortive HIV infection. These findings provide insights into a key host pathway that plays a central role in CD4 T cell depletion during disease progression to AIDS.

HIV-AIDS is a devastating global epidemic with over 70 million infections and 35 million deaths (according to the World

Health Organization). AIDS is primarily caused by loss of the quiescent “bystander” CD4 T cells that populate lymphoid organs. These cells are not permissive for viral replication, which results in abortive infection and the accumulation of incomplete DNA transcripts (*I*). These cytosolic viral DNAs trigger an innate immune response that activates cell death. Here, we sought to identify the host DNA sensor that initiates cell death in abortively infected CD4 T cells.

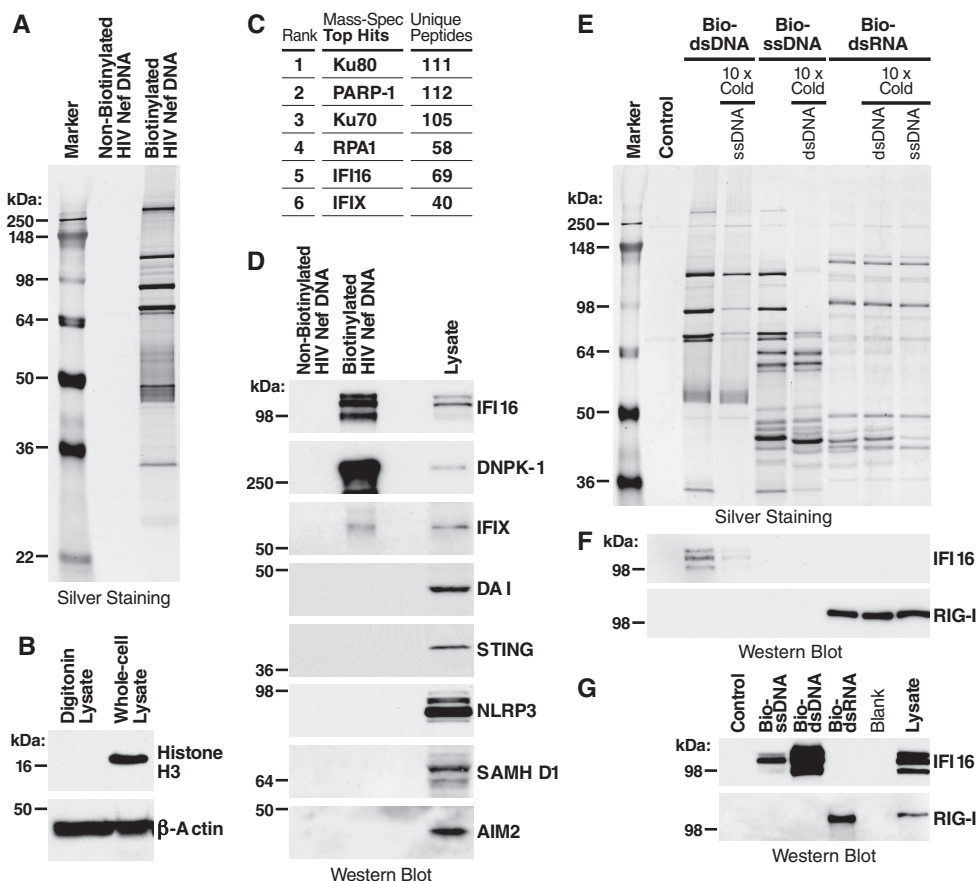
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Fig. 1. Biochemical analysis of cytosolic DNA binding proteins in CD4 T cells. (A)

Tonsillar CD4 T cell lysates were incubated with a 500-bp biotinylated HIV Nef DNA probe or control nonbiotinylated DNA and immunoprecipitated with streptavidin-coated beads. Samples were separated by SDS-PAGE and silver stained. (B) Western blot analysis of nuclear histone H3 and β -actin in whole CD4 T cells or digitonin lysis buffer-prepared CD4 T cell lysates. (C) Top-ranked hits (rank based on protein discriminant scores described in materials and methods) from mass spectrometry samples prepared as in (A). (D) Western blot analysis of candidate DNA sensors. (E) SDS-PAGE and silver stain analysis of biotinylated dsDNA or ssDNA samples prepared as in (A) and competed with a 10-fold excess of ssDNA or dsDNA. (F) Western blot analysis of IFI16 and RIG-I-binding samples in (E). (G) Western blots with high levels of protein input showing IFI16 binding to biotinylated ssDNA and dsDNA and RIG-I-RNA controls.



An unbiased proteomic approach involving DNA affinity chromatography and mass spectrometry was used to identify potential viral DNA sensor candidates. Cytosolic fractions of tonsillar CD4 T cell lysates were incubated with a biotinylated 500-base pair (bp) HIV-1 Nef DNA fragment and subjected to streptavidin immunoprecipitation, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and silver staining (Fig. 1A). The Nef region is reverse-transcribed early; thus, this DNA reverse-transcription product is likely present during abortive HIV infection. Streptavidin immunoprecipitation samples incubated with biotinylated HIV DNA showed numerous bands (Fig. 1A). Nonspecific background binding was very low: Protein was not detected when nonbiotinylated DNA was tested. The cytosolic lysates appeared free of nuclear contamination, as immunoblotting showed no histone H3 (Fig. 1B). Mass spectrometry was used to identify cytosolic proteins from the tonsillar CD4 T cells that bound to HIV DNA. The top six hits, based on protein discriminant scores (27), correspond to Ku80, PARP-1, Ku70, RPA-1, interferon- γ -inducible protein 16 (IFI16), and interferon-inducible protein X (IFIX) (Fig. 1C) (see data file S1 for the complete list).

A rational approach to investigating biologically relevant DNA sensor candidates was pursued in parallel. Expression of various known innate immune sensors was assessed by immunoblotting cytosolic lysates from resting tonsillar CD4

T cells, which confirmed the presence of IFI16 (2, 3); AIM2 (4–7); DAI (8); STING (9–11); DNPK-1 (12); NLRP3 (13–15); and IFIX (PYHIN-1) (16) (Fig. 1D). Cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS) (17, 18) was detected neither at the protein level in tonsillar CD4 T cells (fig. S1D) nor in the affinity chromatography–mass spectrometry experiments (data file S1). We were intrigued with IFI16 because it was identified in both approaches and shown to form an inflammasome (3, 16). Of the known inflammasome DNA sensors, IFI16, but not AIM2, bound HIV-1 DNA (Fig. 1D). Because AIM2 binds DNA in a non-sequence-specific manner, we had expected that AIM2 would be a top candidate, but it was not identified by mass spectrometry (data file S1). IFI16 mRNA levels are five times those of AIM2 mRNA in resting tonsillar CD4 T cells (fig. S1A). Of note, all three IFI16 isoforms were detected in the cytosol and nucleus of primary tonsillar CD4 T cells (fig. S1B).

Reverse transcription of the HIV RNA genome initially generates single-stranded DNA (ssDNA) and then double-stranded DNA (dsDNA);

either might be sensed during abortive infection. A biotinylated dsDNA probe was incubated with cytosolic extracts from tonsillar CD4 T cells with 10-fold excess of unlabeled ssDNA as a competitor (Fig. 1E). IFI16 effectively bound dsDNA (Fig. 1F) as described (2, 19) and was in competition with “cold” ssDNA. Biotinylated ssDNA was subjected to binding and competition with cold dsDNA, but IFI16 was not initially detected by immunoblotting. However, further analysis with higher protein input confirmed that IFI16 binds to ssDNA, albeit more weakly than to dsDNA (Fig. 1G). Retinoic acid-inducible gene I (RIG-I) selectively bound dsRNA as a control (Fig. 1, F and G).

Standard methods, including liposome-mediated delivery of small interfering RNAs (siRNAs) or infection with vesicular stomatitis virus glycoprotein (VSVG)–pseudotyped lentiviruses encoding short hairpin RNAs (shRNAs) are ineffective for targeted gene knockdown in resting CD4 T cells (20, 21). siRNA nucleofection is highly variable, often toxic, and associated with extensive cell death in tonsillar cultures. To overcome these challenges

and to test whether specific DNA sensor candidates are required for cell death in primary lymphoid CD4 T cells undergoing abortive HIV infection, we used an “activation-rest” strategy. Splenic CD4 T cells were activated with phytohemagglutinin (PHA) and cultured in 100 U/ml of interleukin-2 (IL-2), which rendered cells permissive for infection with VSVG-pseudotyped lentiviruses encoding shRNA and mCherry. mCherry-positive cells were isolated by cell sorting (fig. S2), expanded by two rounds of activation with beads conjugated with antibodies against CD3 and CD28, and then rested by reducing IL-2 levels to 10 U/ml for 3 to 4 days (22). IFI16 protein expression was markedly decreased in the mCherry-positive splenic CD4 T cells receiving the lentivirus encoding shIFI16-A compared with cells receiving the lentivirus encoding the control scramble shRNA (Fig. 2A). Next, the rested mCherry-positive CD4⁺ T cells were cocultured with tonsil or spleen CD4 T cells infected with a green fluorescent protein–HIV (HIV-GFP) reporter virus (NLNG1). In cells expressing the scramble shRNA, marked depletion of CD4 T cells occurred (Fig. 2B); this death

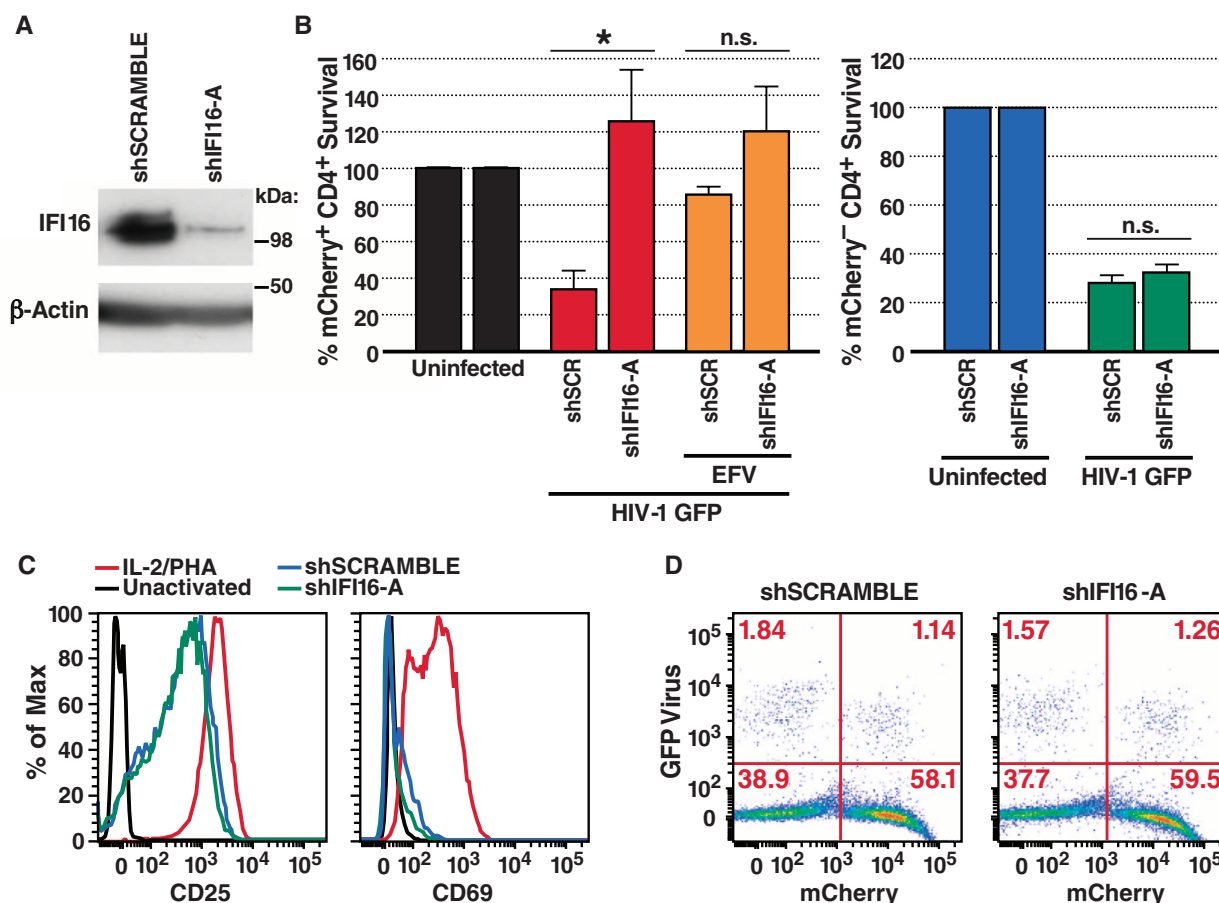


Fig. 2. IFI16 shRNA knockdown rescues activated and rested splenic CD4 T cells from depletion after abortive HIV infection. (A) Western blot analysis of IFI16 and β-actin expression in shRNA-expressing mCherry-positive CD4⁺ T cells after activation and rest in reduced IL-2. (B) Flow cytometric analysis of mCherry⁺ CD4⁺ T cell survival after knockdown with shSCR (scramble shRNA) or shIFI16-A and coculture with either donor-matched mCherry⁻ CD4⁺ T cells or tonsillar HLACs spinoculated with an

HIV-1-GFP reporter virus. Cells were cocultured in the presence or absence of 100 nM efavirenz or with uninfected cells. Data represent the average of three independent experiments from three different donors. Error bars, SEM; **P* < 0.05 (Student's *t* test); n.s., not significant, *P* > 0.05. (C) Flow cytometric analysis of CD25 and CD69 expression after IL-2 reduction. (D) Flow cytometric analysis of mCherry⁺ GFP⁺ populations in shRNA-expressing spleen cells post coculture.

was rescued by adding a nonnucleoside reverse-transcriptase inhibitor, efavirenz (EFV), which implicates abortive HIV infection as previously described (1). In sharp contrast, introduction of shIFI16-A resulted in survival of the mCherry-positive CD4⁺ T cells. In the same experiments, mCherry-negative CD4⁺ T cells were markedly depleted, which suggested that they had returned to a sufficient state of rest to undergo abortive infection.

To exclude more formally the possibility that the “activated and rested” CD4 T cells were dying as a result of productive infection, we assessed the activation status of these cells. Flow cytometric analysis revealed that CD4 T cells cultured in reduced concentrations of IL-2 had lower levels of CD25 and CD69 than cells activated with 100 U/ml IL-2 and 10 μ g/ml PHA (Fig. 2C). However, CD25 levels were higher than found in unactivated cells, which indicated that these cells had not fully returned to a resting state. This finding likely relates

in part to the up-regulation of CD25 expression by IL-2 (23). To directly test how permissive these cells were to productive HIV infection, we utilized an HIV-1–GFP reporter virus. In cells expressing shScramble or shIFI16-A, only ~1 to 2% of the mCherry-positive cells and ~1 to 2% of mCherry-negative cells were productively infected, as indicated by GFP expression (Fig. 2D). Thus, the 60 to 70% depletion of CD4 T cells observed was not due to high levels of productive viral infection.

To confirm IFI16 as an HIV-1 DNA sensor and to test a broader array of potential candidates, we used a second, more rapid shRNA knock-down strategy. Virus-like particles (VLPs) were packaged with the simian immunodeficiency virus (SIV) accessory protein Vpx that degrades the SAMHD1 restriction factor and renders cells susceptible to lentiviral infection (24, 25). This method was adapted for use in resting CD4 T cells on the basis of prior success in cells of myeloid

origin (26, 27). Twenty-four hours after VLP-Vpx spinoculation, complete tonsillar HLACs were spinoculated with shRNA-mCherry lentiviral vectors pseudotyped with HIV glycoprotein 160 (gp160) envelope protein (Env) (fig. S3) (28). Cells were cocultured 3 days later with human embryonic kidney (HEK) 293T cells producing or not producing HIV-1 virions. CD4 T cell death was assessed 1 or 2 days later in mCherry-positive CD4⁺ T cells expressing the shRNA and mCherry-negative CD4⁺ T cells lacking the shRNA. In parallel, EFV was added to select wells.

Three independent shRNAs targeting IFI16 reduced IFI16 protein expression in mCherry-positive CD4⁺ T cells compared to the shScramble control (Fig. 3, A and C). All three IFI16 shRNAs prevented depletion of mCherry-positive CD4⁺ T cells, whereas shScramble did not (Fig. 3, B and D). EFV rescued depletion of scramble-shRNA-expressing cells, which supported the notion that the CD4 T cell depletion resulted from abortive

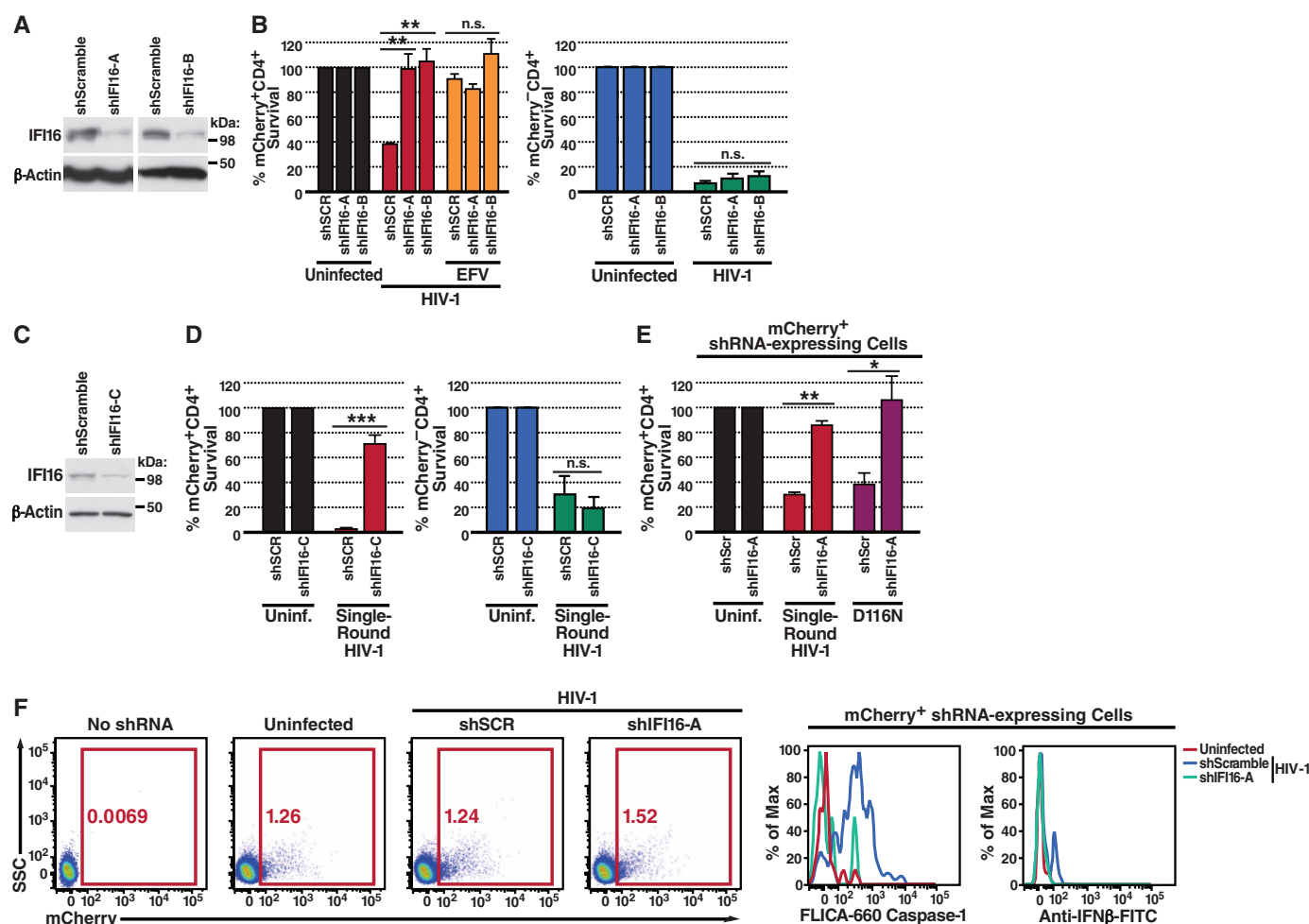


Fig. 3. shRNA knockdown of IFI16 rescues HIV-induced tonsillar CD4 T cell depletion. (A) Western blot analysis of IFI16 and β -actin expression in mCherry⁺ tonsillar CD4 T cells receiving shScramble (shSCR), shIFI16-A, or shIFI16-B. (B) Quantification of flow cytometry of HLACs infected with VLP-Vpx, followed by shSCR, shIFI16-A, or shIFI16-B lentiviruses pseudotyped with HIV gp160 Env then cocultured with HEK293T cells producing HIV-1. $^{**}P < 0.01$ (Student's *t* test); n.s., not significant, $P > 0.05$. (C)

Western blot analysis of shIFI16-C knockdown. (D) Quantification of flow cytometry results as in (B). $^{***}P < 0.001$. (E) Quantification of mCherry⁺ gate of HLACs treated as in (B) with single-round HIV-1 Δ Env pseudotyped with gp160 envelope or HIV-1 D116N integrase mutant. $^{**}P < 0.01$, $^{*}P < 0.05$. (F) Flow cytometric analysis of FLICA-660 caspase-1 and IFN- β intracellular staining in mCherry⁺ cells. Histograms are representative of results obtained from two donors.

infection (Fig. 3B). Moreover, mCherry-negative CD4⁺ T cells were depleted regardless of the shRNA, which demonstrated that experimental conditions were sufficient for abortive infection in all infected samples (Fig. 3, B and D). Thus, using an independent method for shRNA knockdown, we confirmed that IFI16 is required for lymphoid CD4 T cell depletion by HIV after abortive HIV infection.

To confirm that shScramble mCherry-positive CD4 T cells die via abortive infection, which requires reverse transcription but not integration (1), cells were cocultured with HEK293T cells to produce single-round HIV-1 (Δ Env with gp160 coexpressed) or HIV containing a disabling integrase mutation, D116N, in which aspartic acid at codon 116 is replaced by asparagine (Fig. 3E). These replication-defective, nonspreading viruses induced depletion of mCherry-positive CD4 T cells expressing shScramble. In contrast, introduction of shIFI16-A rescued cells from HIV-1-mediated depletion. Thus, neither productive

infection nor HIV integration is required for cell death. Knockdown of IFI16 decreased caspase-1 activation in the mCherry-positive cells, whereas interferon- β (IFN- β) was induced in HIV-infected cells with the shScramble control but not in cells expressing shIFI16-A (Fig. 3F). These findings suggest that IFI16 is required to sense incomplete DNA reverse transcripts that accumulate in abortively infected cells; their accumulation leads to caspase-1 activation, which results in the subsequent death of these cells via pyroptosis (29). IFI16 sensing also leads to IFN- β induction.

Although IFI16 shRNAs effectively rescued death of lymphoid CD4 T cells during abortive infection, other DNA sensor candidates were also evaluated. The VLP-Vpx method was used to render resting lymphoid CD4 T cells permissive to infection with lentiviruses encoding shRNAs directed against AIM2 and STING. Although effective in inhibiting expression of AIM2 and STING protein in human (THP-1 cells) (Fig. 4A),

neither of these shRNAs rescued the mCherry-positive cells from depletion (Fig. 4B). Validated shRNAs targeting IFIX (Fig. 4C) or DNP1K-1 (Fig. 4E) also did not rescue mCherry-positive CD4 T cell depletion (Fig. 4, D and F). Moreover, small-molecule inhibitors of DNP1K-1, Nu7026, and Nu7441 (12) did not rescue cells from abortive infection and pyroptosis (Fig. 4G). These findings, and a recent publication, suggest that DNP1K-1 may play a role in DNA sensing only within the small fraction of cells (5% in tonsil) that are permissive for productive HIV infection and trigger noninflammatory apoptosis (12). In contrast, IFI16 appears to be required to detect abortive infection and induction of highly inflammatory pyroptosis in nonpermissive CD4 T cells (Fig. 4H). These cells form the majority of HIV-1 cellular targets in most lymphoid tissues (95% in tonsillar cultures). Both mechanisms likely contribute to HIV-1-induced AIDS, but at different frequencies determined by the number of permissive

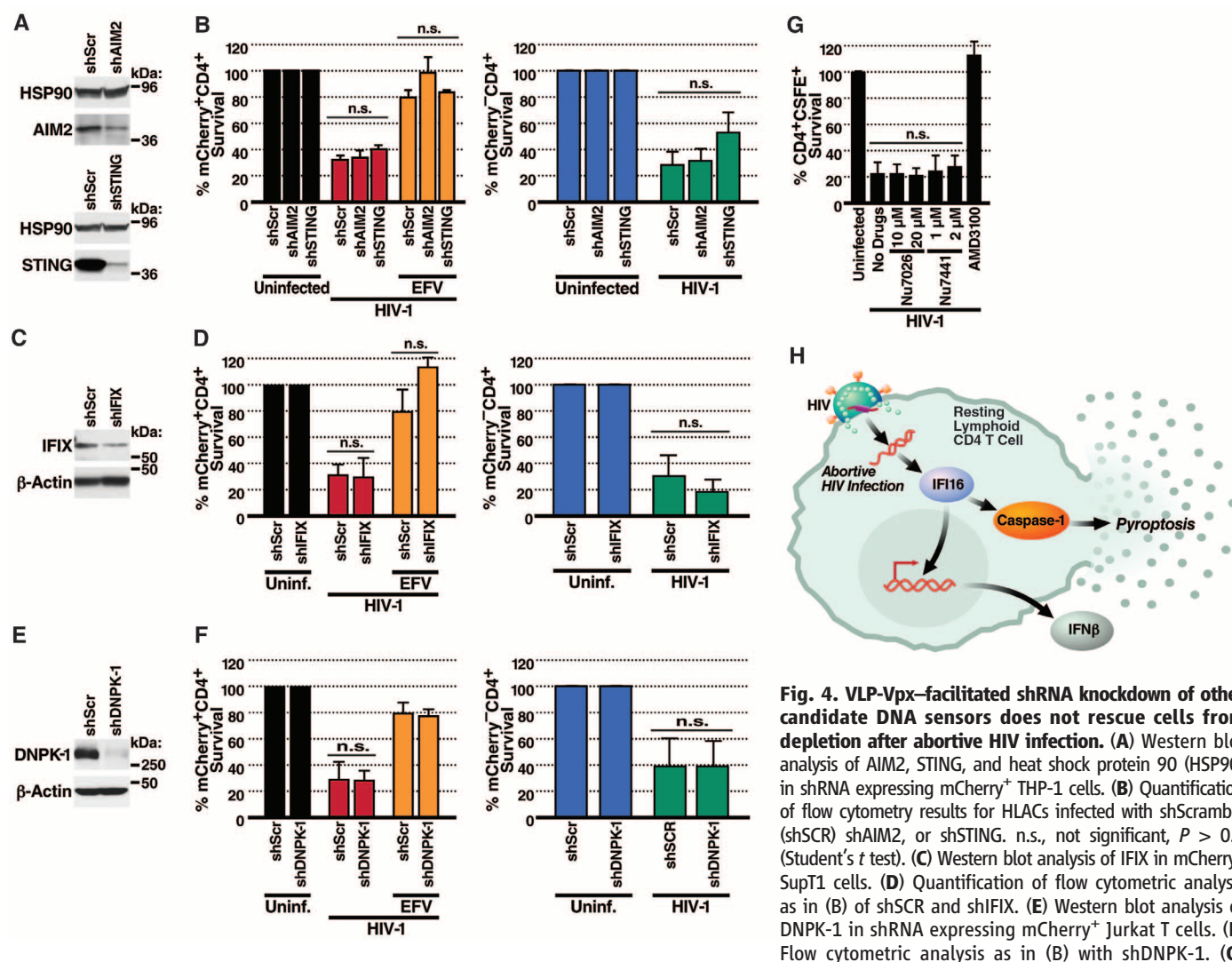


Fig. 4. VLP-Vpx-facilitated shRNA knockdown of other candidate DNA sensors does not rescue cells from depletion after abortive HIV infection. (A) Western blot analysis of AIM2, STING, and heat shock protein 90 (HSP90) in shRNA expressing mCherry⁺ THP-1 cells. (B) Quantification of flow cytometry results for HLACs infected with shScramble (shScr) or shAIM2, shSTING. n.s., not significant, $P > 0.1$ (Student's t test). (C) Western blot analysis of IFIX in mCherry⁺ SupT1 cells. (D) Quantification of flow cytometric analysis as in (B) of shScr and shFIX. (E) Western blot analysis of DNP1K-1 in shRNA expressing mCherry⁺ Jurkat T cells. (F) Flow cytometric analysis as in (B) with shDNP1K-1. (G) Flow cytometric analysis as in (B) with shDNP1K-1. (H) Summary model.

Carboxyfluorescein succinimidyl ester (CFSE)-labeled HLACs were pretreated with dimethyl sulfoxide (DMSO) alone in uninfected and no drug conditions, 10 or 20 μ M Nu7026, or 1 or 2 μ M Nu7441, or 250 nM AMD3100. CFSE⁺ cells were cocultured with donor-matched HLACs productively infected with HIV and analyzed 3 days post coculture. Quantified data represent the average of three independent experiments from three different donors. Error bars, SEM. (H) Summary model.

versus nonpermissive cellular targets residing within various lymphoid tissues.

IFI16 evolved as an antiviral DNA sensor (2, 3). In addition, IFI16 exerts novel antiviral activity, including restriction of herpesvirus replication by inhibiting viral gene expression (30). That IFI16 is targeted for degradation by herpesviruses (31) further highlights an evolutionary pressure to counteract its activity. Our studies reveal that IFI16 initiates an innate immune response that, rather than protecting the host, drives the debilitating CD4 T cell depletion that underlies progression to AIDS in untreated HIV-infected individuals. The cycle of abortive infection, inflammatory death, and recruitment of new cells likely explains how this innate host response is undermined and, in fact, centrally contributes to HIV pathogenesis. Our findings now identify IFI16 as a critical DNA sensor required for cell death during abortive HIV-1 infection. Therapies directed against this host death pathway might preserve CD4 T cells and reduce chronic inflammation—two signature pathologies in HIV infection.

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Acknowledgments: We are grateful for technical assistance from the Gladstone Flow Core, including M. Cavois, M. Gesner,

and J. Tawney. We thank M. Spindler for the generous gift of the pSicoR-mCherry vector, and D. N. Levy for the NLENG1 and D116N plasmids. Efavirenz and azidothymidine were obtained from the AIDS Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH. We thank L. Chavez, I. Munoz-Arias, and N. Byers for technical advice; T. Johnson for technical assistance; G. Howard and A. L. Lucido for editorial assistance; J.C.W. Carroll for graphic arts; and R. Givens and S. Cammack for administrative assistance. The data presented in this manuscript are tabulated in the main paper and the supplementary materials. A patent application has been filed (U.S. provisional serial number 61/511,023) regarding the identification of abortive HIV infection as a driver of CD4 T cell depletion involving the activation by IFI16 of caspase-1 in inflammasomes leading to pyroptosis, an intensely inflammatory form of programmed cell death. This work was made possible with support from the University of California San Francisco–Gladstone Center for AIDS Research (CFAR), an NIH-funded program (P30 AI027763), with additional funding from the Gladstone Institutes. This work was also supported by NIH R21 AI102782, U19 AI096113 (Martin Delaney CARE Collaboratory), and 1DP1036502 to W.C.G. and NIH P50 GM082250, P01 AI090935, and P50 GM081879 to N.J.K. N.J.K. is a Keck Young Investigator. K.M.M. is an A. P. Giannini Fellow supported by the A. P. Giannini Foundation.

Supplementary Materials

www.sciencemag.org/content/343/6169/428/suppl/DC1
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23 July 2013; accepted 4 December 2013
Published online 19 December 2013;
10.1126/science.1243640

Adaptation of Innate Lymphoid Cells to a Micronutrient Deficiency Promotes Type 2 Barrier Immunity

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How the immune system adapts to malnutrition to sustain immunity at barrier surfaces, such as the intestine, remains unclear. Vitamin A deficiency is one of the most common micronutrient deficiencies and is associated with profound defects in adaptive immunity. Here, we found that type 3 innate lymphoid cells (ILC3s) are severely diminished in vitamin A–deficient settings, which results in compromised immunity to acute bacterial infection. However, vitamin A deprivation paradoxically resulted in dramatic expansion of interleukin-13 (IL-13)–producing ILC2s and resistance to nematode infection in mice, which revealed that ILCs are primary sensors of dietary stress. Further, these data indicate that, during malnutrition, a switch to innate type 2 immunity may represent a powerful adaptation of the immune system to promote host survival in the face of ongoing barrier challenges.

Maintenance of barrier defense is an absolute requirement for mammalian host health and survival. Intestinal immunity has evolved in the context of constitutive exposure to microbial pressures. In this regard, the gastrointestinal (GI) tract is home to an estimated 100 trillion commensals, and more than 2 billion humans are chronically infected with parasitic worms (1, 2). Over the course of evolution, maintenance of barrier immunity had to adapt to unstable nutritional availability in the face of

these ongoing challenges. Although dietary ligands can be sensed by immune cells (3–6), it is not clear how the immune system integrates dietary cues in order to tune immune responses on the basis of the nutritional state of the host.

Malnutrition remains the primary cause of immunosuppression worldwide (7). Nonetheless, despite profoundly impaired adaptive immunity associated with malnutrition, most humans can survive for extended periods of severe dietary restriction. We postulated that compensatory

mechanisms might be in place to sustain defined branches of immunity and, in particular, responses associated with the protection of barrier tissues. Vitamin A deficiency is one of the most common nutrient deficiencies, affecting an estimated 250 million children in regions of the world where chronic worm infections are prevalent (8). This essential micronutrient supports adaptive immunity through its metabolite, retinoic acid (RA), which is highly enriched in the gastrointestinal tract (9, 10). Here, we examine the possibility that innate lymphoid cells (ILCs)—potent mediators of barrier maintenance, tissue repair, and host defense (11, 12)—may be primary

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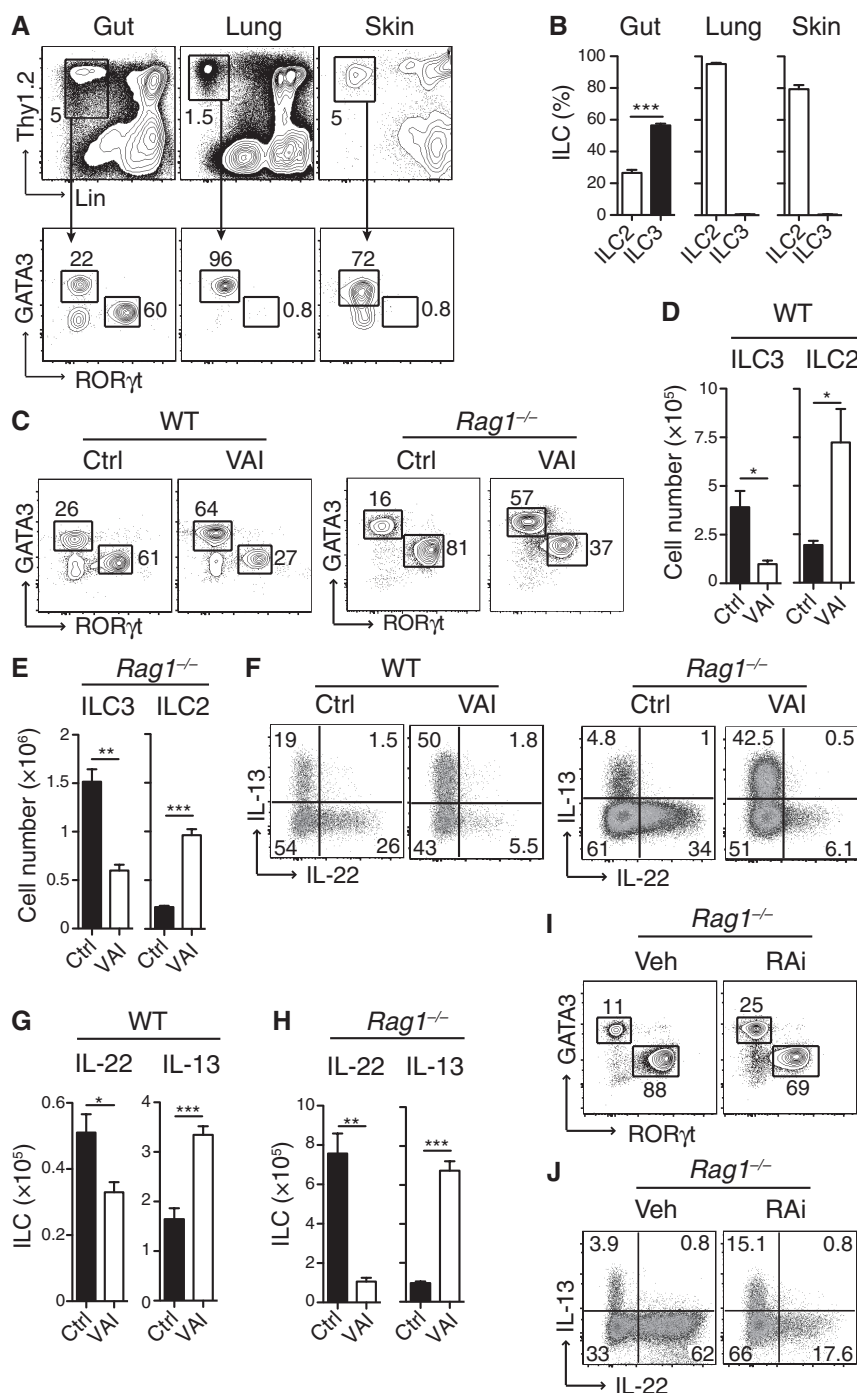


Fig. 1. ILC3s are enriched in the GI tract and depend on RA. (A) Flow cytometric analysis of cells isolated from small intestinal lamina propria (gut), lung, and skin of naïve C57BL/6 mice. (Top) Live CD45⁺ cells stained with Thy1.2 and lineage (lin) markers (NK1.1, T cell receptors TCR β and TCR $\gamma\delta$, CD11b, CD11c, CD4, CD8 α , CD8 β , CD19, GR-1, DX5, and Ter119). (Bottom) Cells gated on Lin⁻ and Thy1.2 expression (ILCs), stained for ROR γ t (ILC3s) and GATA3 (ILC2s). (B) Frequencies of ILC2s and ILC3s in gut, lung, and skin. (C) Small intestinal lamina propria (SiLP) cells from control (Ctrl) or vitamin A-insufficient (VAI) WT or *Rag1*^{-/-} mice, gated on Lin⁻ Thy1.2⁺ cells and analyzed for GATA3 and ROR γ t expression. (D and E) Total numbers of ILC3s (ROR γ t⁺) and ILC2 (GATA3⁺) cells in the SiLP of WT and *Rag1*^{-/-} mice. (F) Intracellular IL-13 and IL-22 expression in Lin⁻ Thy1.2⁺ cells after stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin. (G and H) Total numbers of IL-22- and IL-13-producing ILCs in the SiLP. (I) SiLP ILC2s and ILC3s from *Rag1*^{-/-} mice treated with vehicle control (Veh) or RAi for 8 days. (J) Intracellular IL-13 and IL-22 expression in ILCs after stimulation with PMA and ionomycin. Results are representative of at least three independent experiments with three to five mice in each experimental group. All graphs display means $\pm$ SEM.

sensors of dietary stress able to sustain barrier defense in the context of vitamin A deficiency.

Several subsets of ILCs have been described, with two dominant tissue-resident populations (12). At steady state, retinoic acid receptor (RAR)-related orphan receptor gamma (ROR γ t⁺ ILCs) (ILC3s) are dominantly present in the gut, whereas the majority of ILCs residing in the lung or skin belong to the GATA3⁺ ILC2 subset (11, 13, 14) (Fig. 1, A and B). We explored the possibility that vitamin A metabolites may act as a local cue to control ILC populations. As previously described, T helper cells, T_H1 and T_H17 (15, 16), as well as T_H2 cells, were significantly reduced in the gut of vitamin A-deficient (VAI) mice (fig. S1, A and B). The number of gut resident ILCs, ILC1s, and lymphoid tissue inducer cells (LTi's) remained unchanged in VAI mice (fig. S2, A to D). However, ILC3s and ILC-derived IL-22 and IL-17 were greatly reduced in VAI wild-type (WT) and *Rag1*^{-/-} mice (Fig. 1, C to H, and figs. S3 and S4). Impaired cytokine expression was associated with reduced expression of ROR γ t (fig. S4B). In contrast, we observed a significant increase in ILC2- and ILC-derived IL-13, IL-5, and IL-4 in the gut of VAI mice (Fig. 1, F, G, and H, and fig. S5, A and B).

Acute inhibition of RA signaling with the pan-RAR inhibitor BMS493 (RAi) (17) also resulted in reduced ILC3s and ILC-derived IL-22 and inversely increased ILC2s and ILC-derived IL-13 in both WT and *Rag1*^{-/-} mice (Fig. 1, I and J, and fig. S6, A to D). Alterations in ILC subsets subsequent to RA deprivation occurred independently of commensals (fig. S7). Short-term treatment of VAI *Rag1*^{-/-} mice with RA restored ILC3 frequencies and cytokine production to levels found in control mice and reduced ILC2 numbers (Fig. 2, A to C). VAI *Rag1*^{-/-} mice or mice treated with RAi displayed lower frequencies of Ki67-expressing ILC3s than control mice, whereas the number of Ki67-expressing ILC2s was greatly increased (Fig. 2, D and E). Conversely, addition of RA reversed the proliferative potential of ILCs to frequencies observed in control mice (Fig. 2D). Thus, RA acts as a switch to control a proliferative balance between the two intestinal ILC subsets.

To address whether RA signaling influenced the fate of ILCs, we transferred common lymphoid progenitors (CLPs) to mice devoid of ILCs (*Rag2*^{-/-} γ c^{-/-} mice) (12). Transferred CLPs gave rise to both ILC2s and ILC3s that accumulated in the GI tract (18, 19) (Fig. 2, F and G). Exogenous delivery of RA led to a dominant accumulation of ILC3s in the intestine, whereas inhibition of RA signaling favored ILC2 accumulation (Fig. 2, F and G). Both ILC2s and ILC3s selectively express the nuclear receptor RAR α (20) (fig. S8). We retrovirally transfected progenitors with lymphoid potential obtained from mice carrying WT or floxed alleles of the RAR α gene (RAR α ^{fl/fl}) with the Cre recombinase (fig. S9). Transfer of control progenitors resulted in the preferential accumulation of ILC3s, whereas RAR α -deleted progenitors predominately

gave rise to ILC2s (Fig. 2H). Further, addition of RA impaired ILC2 development from ILC2 progenitors (ILC2Ps), whereas inhibition of RA signaling resulted in increased ILC2 accumulation and IL-13 production in culture (Fig. 2I). These results reveal a cell-intrinsic suppressive role for RA on ILC2 maturation.

We next postulated that some effects of RA may result from a direct action on mature ILCs. Highly purified ILC2s exposed to RA or ILC3s treated with RAI for several days maintained their phenotype; therefore, we argue against an inter-conversion of these two populations (fig. S10, A

to D). As previously reported, RA directly increased IL-22 (20) and ROR γ t expression by mature ILC3s and promoted ILC3 accumulation (fig. S11, A to D). Conversely, RA had a negative impact on ILC2 fitness, whereas treatment with RAI increased their proliferation, numbers, and IL-13 production in both mouse (fig. S12, A to C) and human cells (fig. S13).

We next explored the possibility that vitamin A deficiency may tune the responsiveness of ILC2s to factors contributing to their survival or proliferation (11, 12). Using mice deficient in IL-25, thymic stromal lymphopoietin (TSLP),

or IL-33 receptors allowed us to exclude a dominant role for these factors (figs. S14 and S15). Of note, expression of the IL-7R α , required for ILC development and survival (11, 12), was reduced on ILC2s and ILC2Ps in the presence of RA, whereas the absence of RA signaling increased IL-7R α expression by murine ILC2s and ILC2Ps, as well as human ILC2s (fig. S16, A to E). Increased IL7R α expression conferred enhanced signaling capacity in response to IL-7 as measured by increased signal transducer and activator of transcription 5 (STAT5) phosphorylation (fig. S16F). Blockade of IL-7 signaling in vivo abrogated

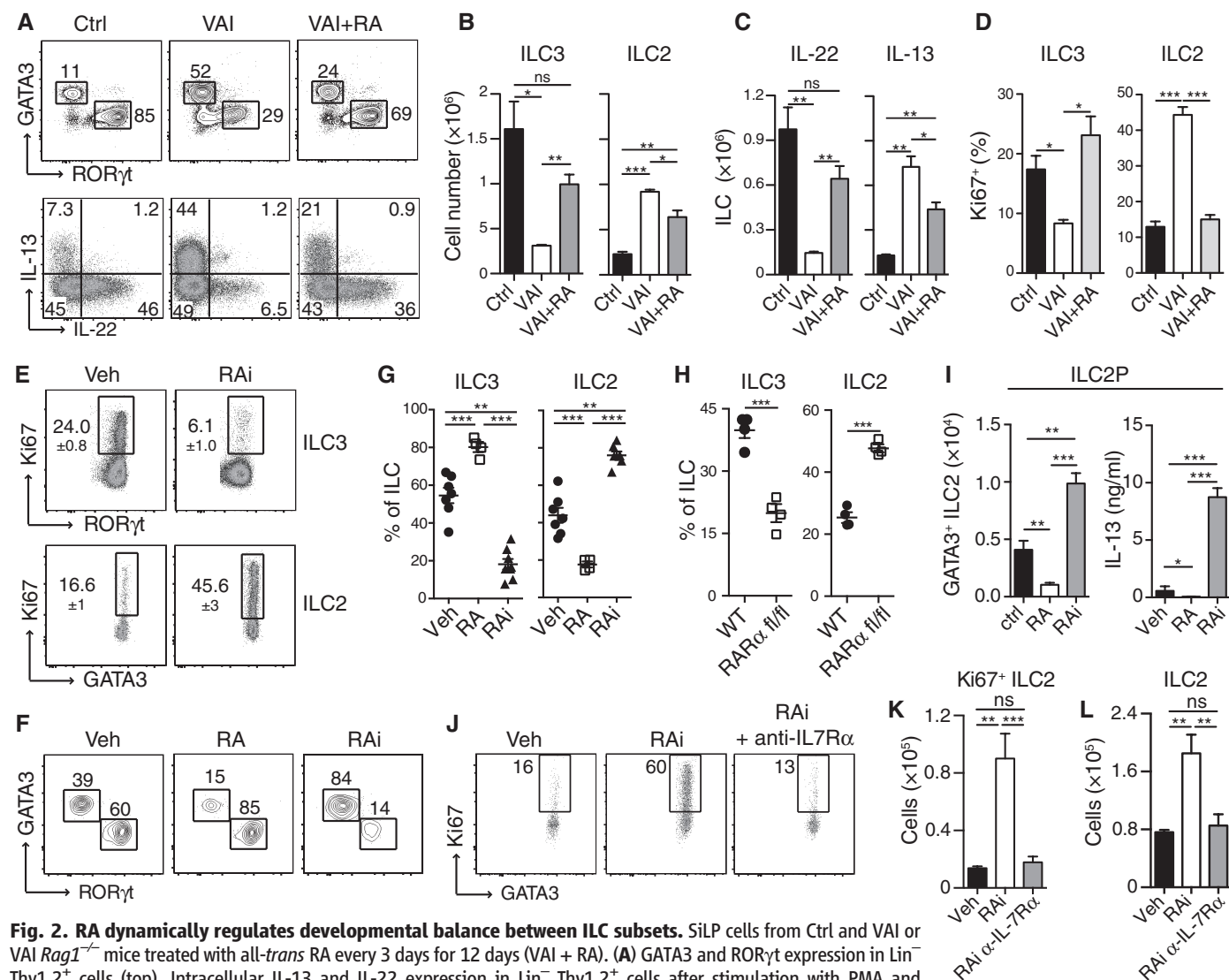


Fig. 2. RA dynamically regulates developmental balance between ILC subsets. SiLP cells from Ctrl and VAI or VAI *Rag1* $^{-/-}$ mice treated with all-*trans* RA every 3 days for 12 days (VAI + RA). (A) GATA3 and ROR γ t expression in Lin $^{-}$ Thy1.2 $^{+}$ cells (top). Intracellular IL-13 and IL-22 expression in Lin $^{-}$ Thy1.2 $^{+}$ cells after stimulation with PMA and ionomycin (bottom). (B) Total numbers of ILC3s and ILC2s in SiLP and (C) total numbers of IL-22- and IL-13-producing ILCs in the SiLP. (D) Frequencies of Ki67 expression in ILC3s and ILC2s isolated from SiLP of Ctrl, VAI, or VAI + RA *Rag1* $^{-/-}$ mice. (E) SiLP cells from *Rag1* $^{-/-}$ mice treated with RAI or Veh for 8 days stained for intracellular Ki67 in ILC3s (top) cells and ILC2 (bottom). (F) CD45.1 $^{+}$ CLPs ($n = 100,000$) were transferred into congenic CD45.2 $^{+}$ *Rag2* $^{-/-}$ γ c $^{-/-}$ mice and treated either with Veh, RA, or RAI. Fourteen days after transfer, SiLP cells were isolated, stained for GATA3 and ROR γ t, and gated on CD45.1 $^{+}$, Lin $^{-}$ and Thy1.2 $^{+}$ cells. (G) Quantification of relative proportion of ILC3s and ILC2s in recipient mice and (H) frequencies of ILC2s and ILC3s from RAR α fl/fl mice. Transferred cells were gated on green fluorescent protein-positive (GFP $^{+}$) cells. (I) ILC2Ps were sort purified from bone marrow and cultured in vitro with IL-7 and SCF in the presence of Veh, RA, and RAI for 7 days. Total numbers of Thy1.2 $^{+}$ GATA3 $^{+}$ cells (left) and IL-13 production in the culture supernatant (right). (J) Ki67 expression in small intestinal ILC2s from mice treated with RAI or Veh and antibody against IL7R α . (K) Total number of Ki67 $^{+}$ ILC2s and (L) total number of ILC2s. Data are representative of three (A to G) or two (H to L) independent experiments with three or four mice in each experimental group or at least two independent experiments with three experimental groups of cells isolated from two mice each (I). (G) displays pooled data from three independent experiments. All graphs display means $\pm$ SEM.

the increase in proliferation and accumulation of ILC2s after RA inhibition but not in control mice (Fig. 2, J to L, and fig. S17). Together these results demonstrate that vitamin A deficiency is associated with altered ILC homeostasis and, in particular, increased IL-13-producing ILC2s, an effect potentially mediated by increased IL-7 responsiveness.

A corollary of our findings is that differential levels of vitamin A could promote different classes of barrier immunity with physiological levels of vitamin A associated with functional ILC3 responses, whereas reduced levels were associated with enhanced innate type 2 immunity. Indeed, both WT VAI mice and mice treated with RAI displayed enhanced susceptibility and pathology to *Citrobacter rodentium* compared with control mice (Fig. 3, A and B), a phenotype associated with impaired T_H17 and ILC3 responses (Fig. 3, C and D, and fig. S18, A to E). Treatment with RAI of *Rag1*^{-/-} mice, in which ILCs are the dominant source of IL-22 (21), dramatically increased pathology and mortality after infection (Fig. 3, E to I), an effect reversed by exogenous delivery of IL-22 (Fig. 3, J and

K). This observation may provide an additional explanation for the profound susceptibility to gastrointestinal bacterial infections observed in children suffering from vitamin A deficiency (22, 23).

Our results, thus far, predict that although T_H1 , T_H17 , and ILC3 collapse under vitamin A deficiency, type 2 immunity might be paradoxically augmented via an enhanced ILC2 response. In support of this, withdrawal of vitamin A heightened mucus production (15). Goblet cells play a central role in barrier protection by the secretion of mucus and antimicrobial peptides (24). Consistent with increased ILC2s observed in the absence of vitamin A, VAI *Rag1*^{-/-} mice displayed significant goblet cell hyperplasia and increased goblet cell-associated expression of the RELM- β gene, *Retnlb*, an effect largely dependent upon IL-13 (Fig. 4, A, B, and C). In these settings, IL-13 production was predominantly ILC2-derived (fig. S19). We next addressed whether enhanced ILC2 responses could compensate for the defect in T_H2 immunity during vitamin A deficiency (25–29). To this end, we used a high dose of *Trichuris muris* eggs associated with T_H2 induc-

tion (30). Although blocking RA impaired T_H2 induction, ILC2 numbers were significantly increased compared with those in infected control mice (fig. S20, A and B). Remarkably, RAI-treated mice controlled parasite burden comparably to control mice, which supports the idea that ILC2s sustained worm control (fig. S20C). Further, both VAI and RAI-treated mice showed enhanced protection to physiological low-dose *T. muris*, a response associated with significant increase in the numbers of ILC2s and IL-13-producing ILCs (Fig. 4, D to H and fig. S21, A and B). In agreement with the role of IL-13 in *T. muris* control (31), accelerated worm clearance was critically dependent on this cytokine (Fig. 4G). We next assessed the role of ILC2s in mediating worm expulsion independently of adaptive immunity. Remarkably, VAI *Rag1*^{-/-} mice displayed enhanced protection and reduced worm burden associated with substantial increase in ILC2 numbers and IL-13 production (Fig. 4, H, I, and J). Thus, vitamin A insufficiency leads to sustained and, in some cases, augmented control of nematode infection via the promotion of ILC2-dependent type 2 immunity.

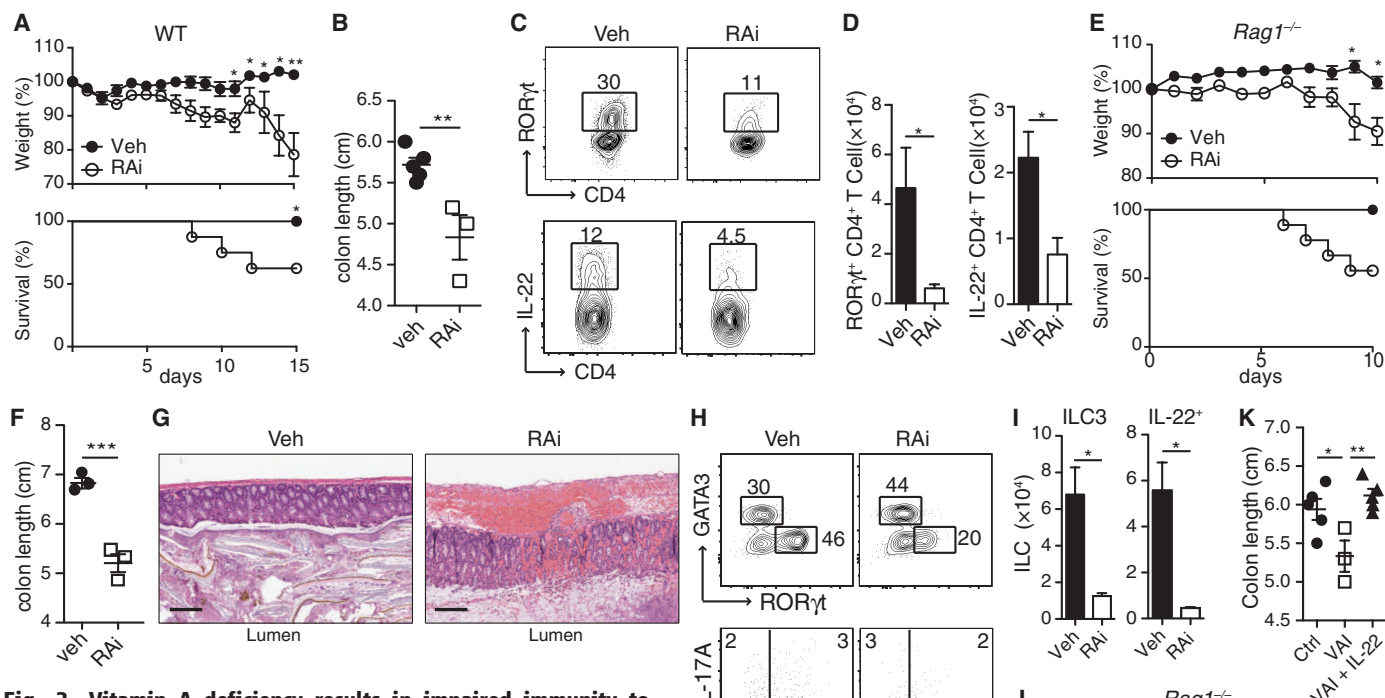


Fig. 3. Vitamin A deficiency results in impaired immunity to bacterial infections. WT (A to D) and *Rag1*^{-/-} (E to K) mice treated with Veh or RAI were infected with *C. rodentium*. (A) Percentile change of original body weight and frequency of surviving animals and (B) colon length of WT mice treated with RAI or Veh. (C) Large intestine lamina propria (LiLP) cells isolated from Veh or RAI-treated WT mice 10 days after infection with *C. rodentium*, gated on CD4⁺ and TCR β ⁺ cells and analyzed for ROR γ t (top) and IL-22 expression (bottom). (D) Total numbers of ROR γ t⁺ and IL-22⁺ CD4⁺ T cells. (E) Percentile change of original body weight and frequency of surviving *Rag1*^{-/-} mice treated with Veh or RAI and infected with *C. rodentium*. (F) Colon length and (G) representative hematoxylin and eosin (H&E)-stained histological sections of colonic tissue analyzed 10 days post infection; scale bars, 200 μ m. (H) LiLP ILC2s and ILC3s from Veh or RAI-treated *Rag1*^{-/-} mice 10 days after infection with *C. rodentium* (top) and intracellular IL-17A and IL-22 expression in ILCs after stimulation with PMA and ionomycin (bottom). (I) Total numbers of ROR γ t-expressing ILCs and total numbers of IL-22-producing ILCs per colon. (J) Weight loss of Ctrl, VAI, or VAI *Rag1*^{-/-} mice treated with IL-22 (VAI+IL-22) and infected with *C. rodentium*. (K) Colon length analyzed 14 days post infection. Data represent at least two independent experiments with three to five mice in each experimental group. All graphs display means $\pm$ SEM.

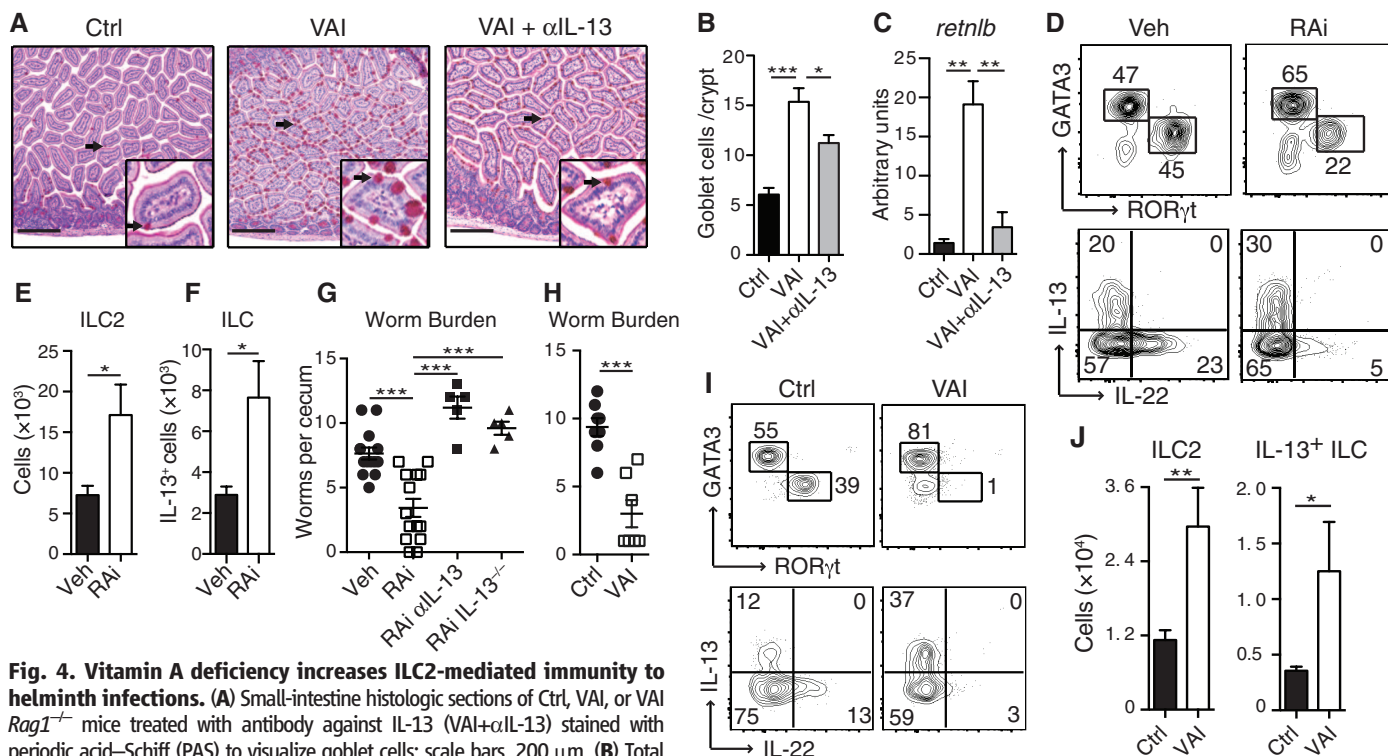


Fig. 4. Vitamin A deficiency increases ILC2-mediated immunity to helminth infections. (A) Small-intestine histologic sections of Ctrl, VAI, or VAI *Rag1*^{+/+} mice treated with antibody against IL-13 (VAI+αIL-13) stained with periodic acid–Schiff (PAS) to visualize goblet cells; scale bars, 200 μm. (B) Total numbers of PAS-positive goblet cells per crypt and (C) *Retnlb* (RELM-β) gene expression in the small intestine of Ctrl, VAI, or VAI+αIL-13 *Rag1*^{+/+} mice. (D) Lamina propria ILC2s and ILC3s isolated from the cecum of Ctrl or VAI WT mice 13 days after oral infection with *T. muris* (top) and intracellular IL-13 and IL-22 expression in ILCs after stimulation with PMA and ionomycin (bottom). (E) Total numbers of ILC2s and (F) total numbers of IL-13–producing ILCs in the cecum. (G) Worm burden in the cecum of Veh, RAi, RAi-treated IL-13^{-/-} (RAi IL-13^{-/-}), and RAi mice treated with neutralizing antibody against IL-13 (RAi αIL-13) 12 days after infection. (H) Number

of worms in Ctrl and VAI *Rag1*^{+/+} mice 12 days after infection and (I) intracellular GATA3 and RORγt (top) and IL-13 and IL-22 expression (bottom) in cecal ILCs. (J) Total numbers of ILC2s and IL-13⁺ ILCs in the cecum of *T. muris*–infected mice. Data represent at least two (A and B and H to J) or three (D to G) independent experiments with three to five mice in each experimental group. Data in (G) Veh and RAi is pooled from three experiments, RAi αIL-13 and RAi IL-13^{-/-} represent one experiment each. All graphs display means ± SEM.

Our work suggests that vitamin A and its metabolite RA function together as a dietary alarm signal that allows the host to immunologically respond to its nutritional state. Contrary to the current paradigm, we show that nutrient deficiency is not associated with global immunosuppression but rather can selectively activate a distinct arm of barrier immunity. Notably, we found that ILC2s act as primary sensors of dietary stress able to compensate for the collapse of adaptive immunity in settings of nutrient deprivation. Type 2 immunity and, in particular, IL-13 are associated with tissue repair, increased mucus production, and physiological responses all aimed at reinforcing barrier integrity and defense (32, 33). Furthermore, enhanced type 2 responses are clearly beneficial in the context of exposure to worms that have been partners throughout human evolution and still represent the major form of parasitic infection worldwide. Because nematodes compete with the host for nutritional resources, reinforcement of antihelminth immunity can also provide a substantial advantage to the host. Thus, in settings of malnutrition, a rapid switch to type 2 barrier immunity imposed by vitamin A deficiency may represent a powerful adaptation of the immune system to transiently promote host survival in the face of dominant barrier exposures.

Such a strategy leaves the host vulnerable to potential encounters with acute diarrheal pathogens but could provide a survival strategy to temporarily reduce the pressure from its constitutive evolutionary partners, worms and commensals, in settings of nutritional deprivation.

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Acknowledgments: This work was supported by the Division of Intramural Research of the National Institute of Allergy and Infectious Diseases (NIAID), NIH; Office of Dietary Supplements, NIH; NIH grant F30 DK094708 (S.P.S.); Human Frontier Science Program (C.W.); U.S. Department of Agriculture–Agricultural Research Service project plan #1254-32000-094-00D (J.U.); and by the Damon Runyon Cancer Research Foundation (Dale F. and Betty Ann Frey Fellow, J.A.H.). We thank the NIAID animal facility staff; K. Holmes and the NIAID sorting facility, in particular, C. Eigsti

and E. Stregovsky; and K. Becht and the NIAID gnotobiotic facility, in particular, C. Avecedo and D. Trageser-Cesler for technical assistance. We thank D. Artis for providing *C. rodentium*, W. J. Leonard for providing *TSLPR*^{-/-} mice, and W. Paul for providing *IL33R*^{-/-} mice. We thank the Belkaid lab for critical discussions regarding the manuscript.

The data presented in this manuscript are tabulated in the main paper and the supplementary materials.

Supplementary Materials

www.sciencemag.org/content/343/6169/432/suppl/DC1
Materials and Methods

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References (34–39)

24 October 2013; accepted 27 November 2013
10.1126/science.1247606

Transmissible Dog Cancer Genome Reveals the Origin and History of an Ancient Cell Lineage

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Canine transmissible venereal tumor (CTVT) is the oldest known somatic cell lineage. It is a transmissible cancer that propagates naturally in dogs. We sequenced the genomes of two CTVT tumors and found that CTVT has acquired 1.9 million somatic substitution mutations and bears evidence of exposure to ultraviolet light. CTVT is remarkably stable and lacks subclonal heterogeneity despite thousands of rearrangements, copy-number changes, and retrotransposon insertions. More than 10,000 genes carry nonsynonymous variants, and 646 genes have been lost. CTVT first arose in a dog with low genomic heterozygosity that may have lived about 11,000 years ago. The cancer spawned by this individual dispersed across continents about 500 years ago. Our results provide a genetic identikit of an ancient dog and demonstrate the robustness of mammalian somatic cells to survive for millennia despite a massive mutation burden.

Canine transmissible venereal tumor (CTVT) is a naturally occurring transmissible cancer. It is a clonal cell lineage that spreads within the domestic dog population by the allogeneic transfer of living cancer cells, usually during coitus. The disease manifests itself with the appearance of tumors most often associated with the external genitalia of male and female dogs. The first known report of CTVT was made in 1810, when it was described by a London veterinary practitioner as “an ulcerous state, accompanied with a fungous excrescence” that arises in “organs concerned in generation” (1). It has subsequently been reported in dog populations worldwide (2, 3) and is, to our knowledge, the oldest and most widely disseminated cancer in the natural world.

We sequenced the genomes of two CTVT tumors, collected in Maningrida, Australia (24T), and Franca, Brazil (79T), as well as the genomes of their respective hosts 24H, an Aboriginal camp dog, and 79H, an American cocker spaniel (Fig. 1A). We also prepared metaphases for cytogenetic analysis from two CTVTs collected in Cape Verde and Italy. Metaphase fluorescence in situ hybridization (FISH) using red fox chromosomes as probes revealed massive karyotypic rearrangement in the CTVT genome, which was highly consistent between the two tumors analyzed (Fig. 1B). Despite the aneuploidy in CTVT detected by using cytogenetics, a copy-number analysis revealed that the genome is largely diploid [including a large proportion of the genome that is diploid with loss of heterozygosity (LOH)] and that there has been minimal change in copy-number status in 24T and 79T lineages since their divergence (Fig. 1C and tables S1 and S2). In contrast to human tumors, most of which contain several detectable subclones (4), presumably because of positive selection for newly acquired mutations conferring selective advantage (5), we found no evidence for subclonality in CTVT metaphases or copy-number plots. This suggests that CTVT is not undergoing positive selection at high frequency, possibly indicating that it is well adapted to its niche.

CTVT's massive burden of karyotypic abnormalities despite its largely diploid genomic copy number indicates that the genome has undergone large-scale copy neutral structural rearrangement. We found 2118 candidate somatic structural var-

iants that were shared between 24T and 79T and 216 and 72 candidate somatic structural variants in 24T and 79T, respectively, that were unique to one tumor. We also searched for evidence of transposon mobilization in CTVT. We found 348 and 352 transposon insertions (involving both long interspersed nuclear elements and short interspersed nuclear elements) that were unique to 24T and 79T, respectively, and are thus likely to represent somatic retrotransposition events that occurred after the divergence of 24T and 79T.

We identified 3.04 million substitution variants in 24T and 2.77 million in 79T after removing all single-nucleotide polymorphisms (SNPs) known to segregate in the dog or the wolf germ line (including those identified in 24H or 79H). These variants will include somatic mutations as well as SNPs that have not been captured in previous canine sequencing efforts. We estimated the true number of somatic substitution mutations in CTVT by calculating the ratio of homozygous to heterozygous known SNPs in diploid regions that retain both parental chromosomes. Assuming that all homozygous variants in these regions are SNPs, we were able to estimate the number of unannotated heterozygous SNPs in each diploid segment by using the homozygous-to-heterozygous ratio among annotated SNPs. This analysis indicated that at least 65% of unannotated variants in CTVT are likely to be somatic, corresponding to a total of ~1.9 million somatic mutations in CTVT. A total of 103,667 and 109,119 variants were unique to 24T and 79T, respectively, the majority of which probably arose as somatic mutations after the two tumors' divergence because only 2056 and 5647, respectively, of these occur in regions that have been lost in the other tumor. Although a range of total mutation counts is observed in human cancers, the majority have between 1000 and 5000 somatic single-base-substitution mutations (6). Thus, CTVT has acquired several hundred times more somatic mutations than most human cancers.

To ascertain the processes responsible for the mutations in CTVT, we characterized CTVT's mutational spectrum and searched for known mutational signatures (6) in the CTVT genomes. The CTVT mutation spectrum was dominated by C>T (or G>A) mutations and CC>TT (or GG>AA) dinucleotide mutations (Fig. 2, A and B). Four mutational signatures were identified in CTVT (labeled A to D, Fig. 2C), which were sufficient to explain 98% of the mutations in CTVT (0.96 Pearson correlation between the mutation set observed in CTVT and the mutation set reconstructed by using four mutational signatures). The chemical events associated with some of these mutational signatures have been

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characterized. Signature A is associated with germline SNPs, and its contribution to CTVT variant sets probably reflects incomplete removal of germline-inherited variants. Signature B defines the mutational signature characterized by C>T at CpG dinucleotides that is widely found in human cancers and known to be correlated with patient age (6). Signature C [known as signature

5 in (6)] is also frequently present in a spectrum of human cancers; however, its etiology is unknown (6). Signature D, which is characterized by C>T and CC>TT mutations and in humans is predominantly observed in cancers of the skin and known to be associated with exposure to ultraviolet light (6), explains 42% of the mutations in CTVT. These observations suggest that CTVT

has been exposed at a low level to ultraviolet light during its evolution. Although CTVT tumors usually occur inside the genital orifice, they may be exposed to sunlight when they protrude from the vulva, ulcerate through preputial skin, or occur on external surfaces such as the skin or conjunctiva (for example, see 24T and 79T, Fig. 1A). Furthermore, it is the very cells that are exposed

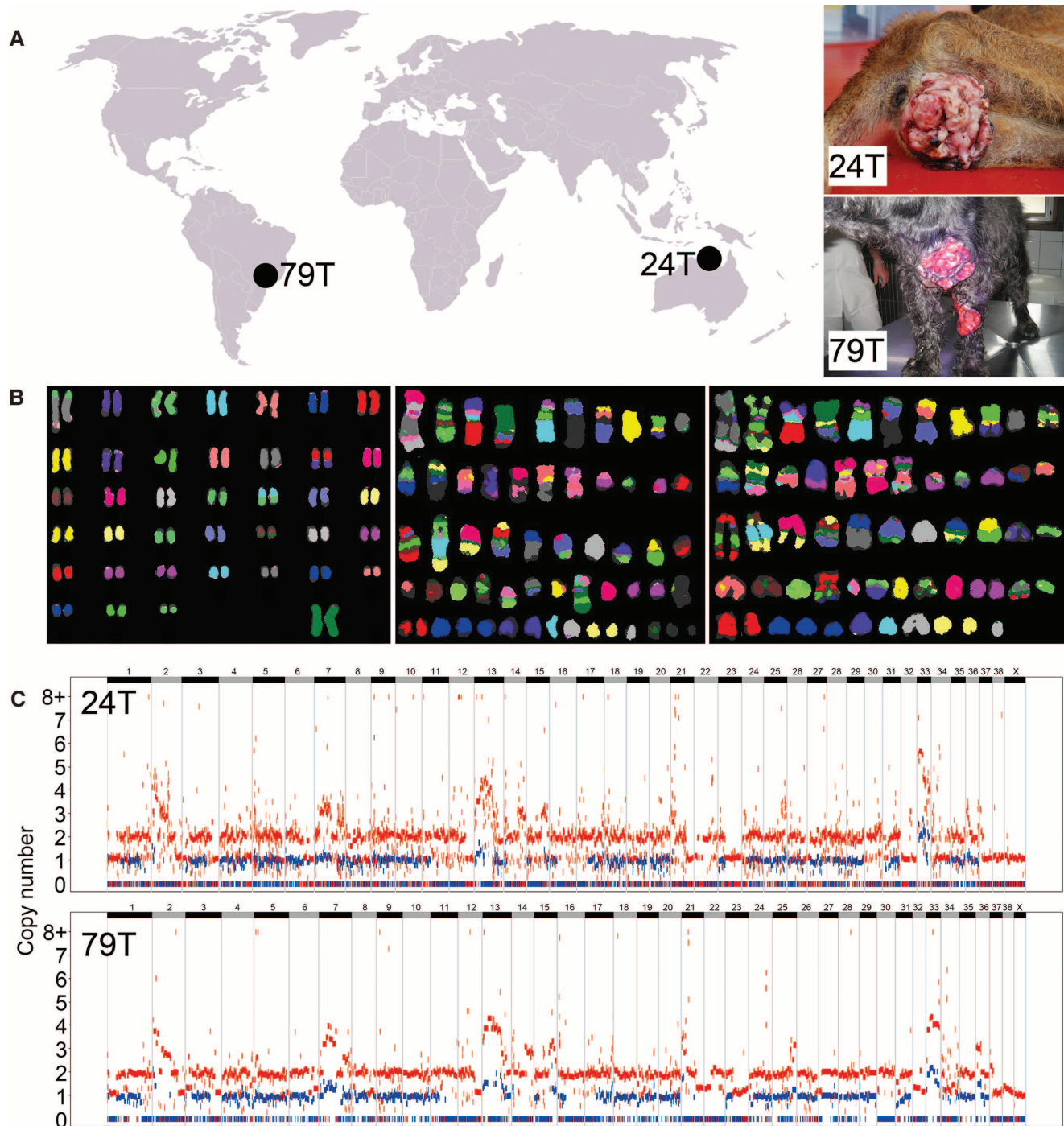


Fig. 1. CTVT tumors, karyotypes, and copy number. (A) Samples sequenced in this study. Both tumor (24T, 79T) and host (24H, 79H) DNA was sequenced from the two individuals shown. (B) Multiplex FISH using red fox probes to investigate karyotypes of a normal female dog (left) and CTVTs collected in Cape Verde (center) and Italy (right). (C) CTVT genomic copy

number for 24T (top) and 79T (bottom). Red and blue points represent total copy number and minor copy number (i.e., copy number of the allele present in fewer copies), respectively, calculated by using normalized read counts at each of 2,544,508 SNP loci. Chromosomes are represented by horizontal alternating black and gray bars.

on the surface of a tumor that are most likely to propagate the CTVT lineage by passage to new hosts.

More than 10,000 genes (10,955 in 24T and 10,546 in 79T) in CTVT carry at least one non-synonymous substitution variant that is not a known germline SNP. Table S3 lists those genes that we consider to be the highest-confidence driver mutations in CTVT. These include a known rearrangement involving *MYC* (7), a homozygous deletion of *CDKN2A*, a hemizygous nonsense mutation in *SETD2*, and a rearrangement involving *ERG* that creates a potential in-frame *NEK1-ERG* fusion gene. A census of genes that have been lost in CTVT by homozygous deletion or hemizygous nonsense mutation indicated that at least 646 genes, 2.8% of the 22,874 protein-coding genes annotated in the dog genome, are collectively dispensable for survival and proliferation of a somatic cell (table S4).

We next sought to reconstruct the phenotype of the CTVT founder animal and to estimate the age of the cancer that it spawned by using the variants found in the CTVT genome. We compared the genotypes of 24T and 79T as well as 24H and 79H at 23,782 polymorphic SNP loci with those of 1106 previously genotyped dogs, wolves, and coyotes (8, 9). The result, displayed by using principal components analysis (Fig. 3A), indicates that the CTVT founder animal was likely to have been a dog belonging to one of the ancient breeds [previous analyses were unable to distinguish between a wolf or an ancient-breed dog origin (10)]. Analysis of a pairwise distance tree indicated that, of the 86 breeds included in the analysis (9), the CTVT founder animal clusters most closely with Alaskan malamutes and

huskies (11 Alaskan malamutes have >95% probability after resampling genotypes of having one of the 16 genotypes closest to CTVT) (Fig. 3B and table S5). As expected, 79H clustered most closely with cocker spaniels within the modern breeds (>95% probability after resampling that each of the six closest genotypes are English cocker spaniels) (Fig. 3, A and B, and table S5). Host 24H, an Aboriginal camp dog, appears to have genetic contributions from both ancient and modern breeds (Fig. 3, A and B, and table S5).

Recent studies have mapped several loci conferring canine phenotypic features, such as coat color, morphology, and behavior (11–21). We examined the sequence of CTVT at a number of these loci to determine the likely phenotype of the founder animal (table S6). Our analysis indicated that the founder animal was likely to have been of medium or large size with an agouti or solid black coat. It carried a mixture of wolflike and doglike alleles at loci that have been linked to dog domestication (21). Tumors 24T and 79T each carried a single X chromosome and had no evidence of a Y chromosome, as found in a previous analysis (22). This is consistent with either a male (after somatic Y chromosome loss) or a female (after somatic X chromosome loss) founder animal (Fig. 3C). Analysis of genome-wide heterozygosity indicated that the founder animal was relatively inbred (Fig. 3D).

Previous studies have estimated that CTVT is between 200 and 70,000 years old (10, 23). We sought to clarify the age of CTVT by using the mutations associated with mutational signature B (Fig. 2C), which is correlated with patient age at diagnosis in many human cancer types (6, 24). We estimated that 492,533 mutations in CTVT are

likely to have been caused by this mutational process. By using the mutation rate of signature B in human medulloblastoma as a molecular clock [43.3 mutations of this signature genome-wide per year; we chose medulloblastoma because it is the human cancer with the closest correlation between number of mutations of this signature and patient age (6)], we estimate that CTVT may have first arisen about 11,368 years ago (lower and upper confidence intervals are 10,179 and 12,873 years, respectively). There is uncertainty in this estimate introduced by the possibilities that the accumulation of mutations of this signature is not clocklike in CTVT and that there are tissue- or species-specific differences in the rate of mutation accumulation between CTVT and human medulloblastoma. Applying this molecular clock to the mutations that occurred after divergence of the two tumors, we suggest that the most recent common ancestor of 24T and 79T may have existed about 460 years ago (458.2 years for 24T, 459.8 years for 79T) (Fig. 3E). We note that the estimated timing of this divergence coincides with the era of rapid human global exploration.

The founder animal whose somatic cells first gave rise to CTVT was an ancient-breed dog that may have lived about 11,000 years ago. The date of CTVT emergence, together with the structure of its phylogenetic tree (23) and evidence for both wolflike and doglike alleles at loci associated with domestication, is consistent with the possibility that CTVT may have first arisen within a genetically isolated population of early dogs whose limited genetic diversity facilitated the cancer's escape from its hosts' immune systems. Similarly, the Tasmanian devil facial tumor disease, the only other known naturally occurring

Fig. 2. CTVT mutations. Analyses were performed on a set of 395,306 CTVT variants that were annotated as somatic because of their heterozygous status within genomic regions that have undergone both LOH and duplication. (A) Simple mutation spectrum in CTVT. Mutations are labeled in pyrimidine context. (B) Dinucleotide mutation spectrum in CTVT. The first base was defined as the mutation with the lower chromosome coordinate, and the second base is immediately adjacent to the first base on the same strand. The strand is displayed relative to the pyrimidine context of the first base. A total of 3518 dinucleotide mutations were included in the analysis. (C) The proportion of mutations in CTVT explained by mutational signatures A to D.

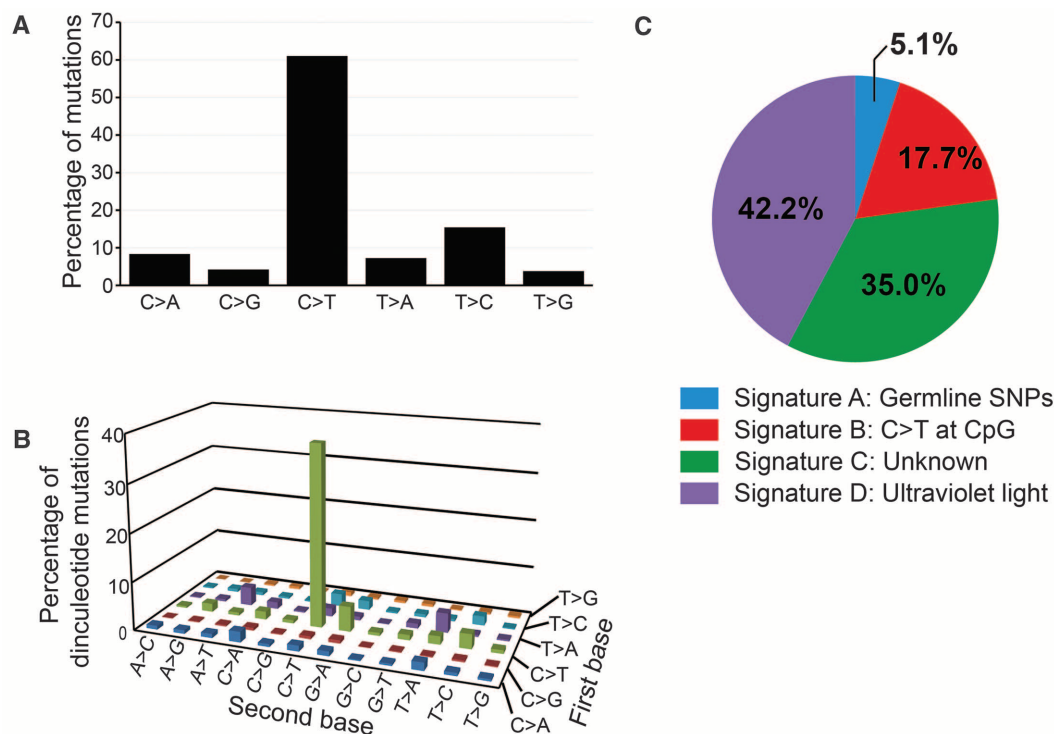
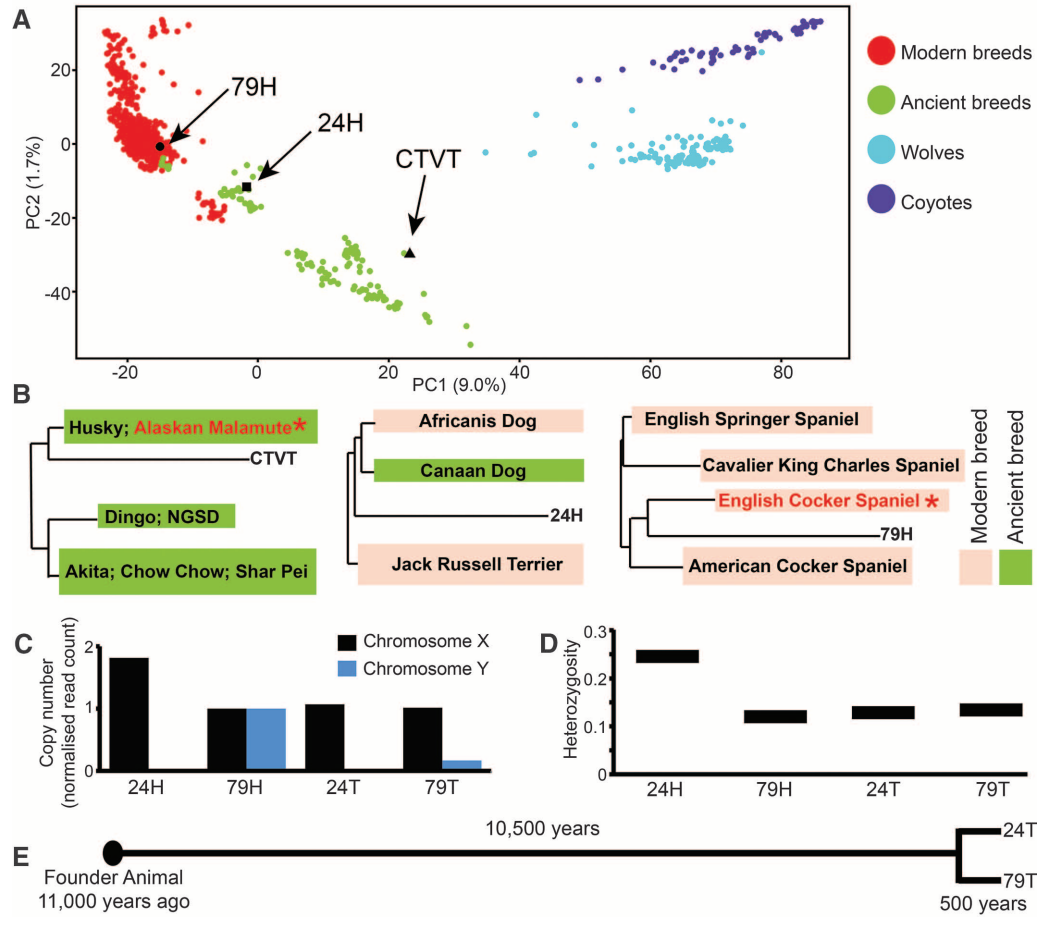


Fig. 3. Tracing the CTVT founder animal.

(A) Principal components (PCs) analysis of 1106 wolves, dogs, and coyotes using genotypes at 23,782 polymorphic SNP loci (8, 9). Each individual is represented by a single colored dot, and positions of CTVT (inferred from the genotypes of 24T and 79T), 24H, and 79H are indicated. Breeds were classified as modern or ancient according to (27). **(B)** Positions of CTVT (left), 24H (center), and 79H (right) on pairwise distance tree comparing genotypes at 23,782 SNP loci with 1106 other dogs, wolves, and coyotes (8, 9). Only the closest breeds to CTVT, 24H, and 79H are shown. Breeds containing members that most strongly clustered with CTVT and 79H after genotype resampling are marked with red text and asterisks (see table S5; 24H did not cluster strongly with individuals from any single breed). NGSD, New Guinea singing dog. **(C)** Sex chromosome copy number of 24H (a female dog), 79H (a male dog), 24T, and 79T determined by counting the number of reads aligning to X and Y chromosomes and normalized to 79H. Y chromosome reads in 79T are likely to be derived from contaminating host DNA. **(D)** Proportion of annotated SNP loci in germline diploid regions that are heterozygous in 24H, 79H, 24T, and 79T. **(E)** Timeline for CTVT origin and divergence.



clonally transmissible cancer, arose in an island population with low genetic diversity (25, 26). Populations with limited genetic diversity may be particularly susceptible to the emergence and spread of transmissible cancers.

The CTVT genome has illuminated the origins, history, and evolution of the world's oldest known cancer. It is remarkable that a somatic genome whose DNA would normally have survived for no more than 15 years during the life of one dog has continued to exist for several millennia as a parasitic life form. CTVT's survival and global dominance are a testament to the ability of the mammalian somatic cell genome to adapt to and persist in a new ecological niche.

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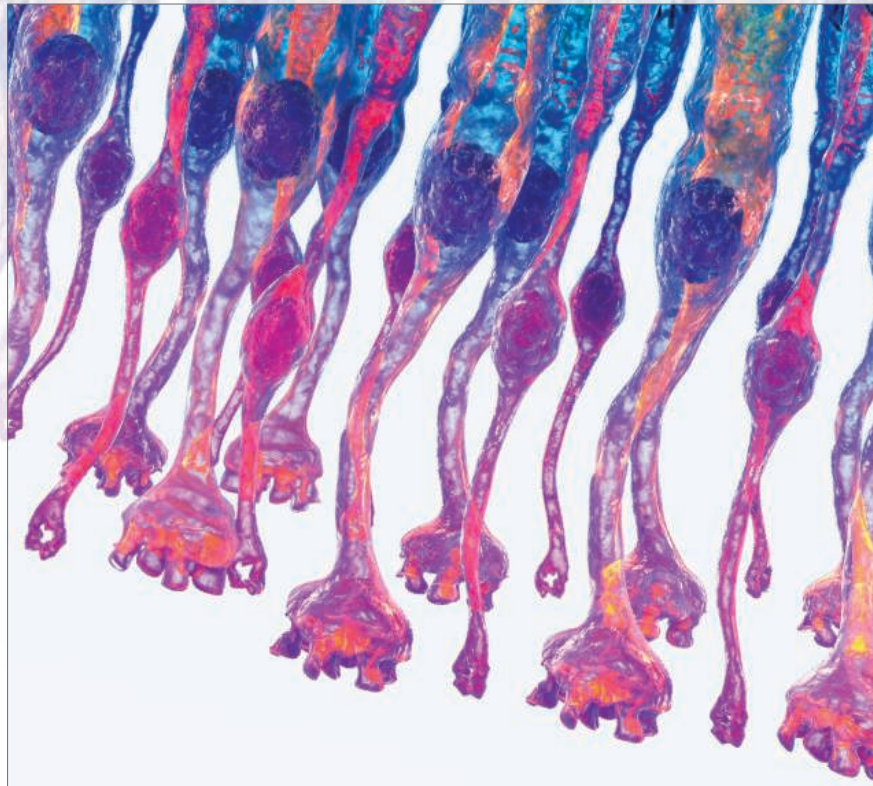
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Acknowledgments: This work was supported by the Wellcome Trust (grant reference 098051), the Kadoorie Charitable Foundation, and a L'Oreal–United Nations Educational, Scientific, and Cultural Organization for Women in Science Fellowship (E.P.M.). We are grateful to A. King, S. Cooke, A. Strakova, M. Peleteiro, C. Semedo, T. M. Morata Raposo, R. R. Huppes, C. Marchiori Bueno, and the people of Maningrida. We thank members of the Wellcome Trust Sanger Institute Cancer Genome Project IT group and the Wellcome Trust Sanger Institute Core Sequencing and IT facilities. Additional sources of support included European Molecular Biology Organization Long Term fellowships [Lt-456-2010 (E.P.M.) and ALTF-1287-2012 (I.M.)] and a Marie Curie Intra-European Fellowship grant (J.M.C.T.). Genome sequence data reported in this study are available with accession number PRJEB5068 in the European Nucleotide Archive.

Supplementary Materials

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Materials and Methods
Tables S1 to S8
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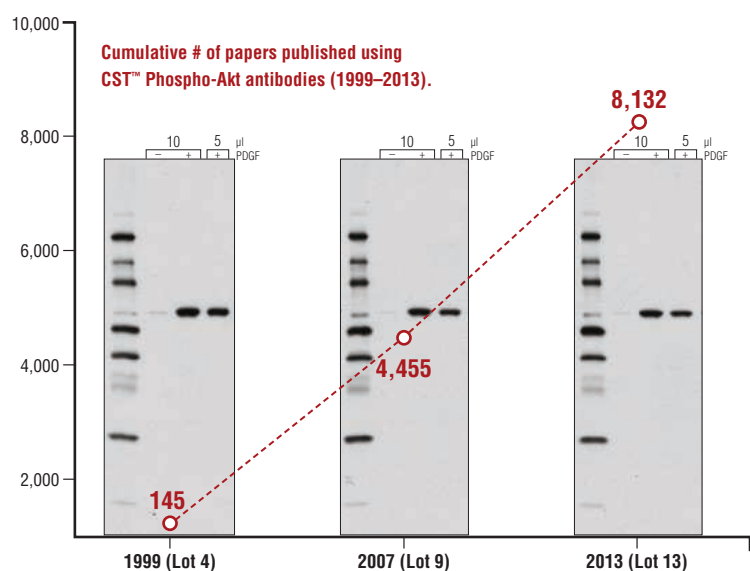
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DIRECTOR Arkansas Children's Nutrition Center

We are seeking to recruit a new Director of the Arkansas Children's Nutrition Center (ACNC) in Little Rock, AR. The Director will be a Professor (Tenure Track) in the Department of Pediatrics and Chief of the Division of Developmental Nutrition at the University of Arkansas for Medical Sciences (UAMS) College of Medicine.



Requirements include a PhD or MD degree and a national reputation in nutrition-related research. He/she should have demonstrated excellence in research as evidenced by accomplishments, such as a long-standing record of nationally competitive funding at the R01 level through NIH; an excellent record of publications in high quality peer-reviewed scientific journals; NIH Study Section Membership; and National Awards. The successful candidate must also have proven and effective management and interpersonal skills necessary to direct a large, interdisciplinary national human nutrition research program.

The ACNC is one of six Human Nutrition Research Centers funded by the USDA/ARS and is housed in a private research building on the campus of Arkansas Children's Hospital, which is one of the nation's largest state-of-the-art children's hospitals and the primary pediatric research and teaching site of UAMS. The position carries a competitive salary and benefits package and will remain open until February 15, 2014.

Interested parties should submit their curriculum vitae and full contact information via email to RFJ@uams.edu or mail to: **Richard Jacobs, MD, FAAP, Search Committee Chair, 13 Children's Way Slot 842, Little Rock, Arkansas 72202-3591.**

UAMS is an inclusive Equal Opportunity and Affirmative Action Employer and is committed to excellence.



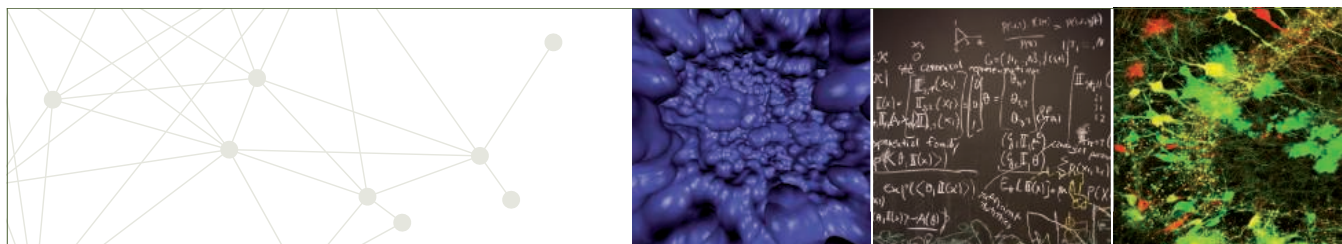
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UChicago Argonne

Director, Argonne National Laboratory

Argonne National Laboratory, managed by UChicago Argonne, LLC under a prime contract with the Department of Energy (DOE), is one of the nation's largest federally-funded research and development centers. Argonne is a multidisciplinary science and engineering institution, where "dream teams" of world-class researchers work alongside experts from industry, academia and other government laboratories to address vital national challenges in clean energy, environment, technology and national security. Widely recognized for its excellence in connecting basic research to innovative technology, Argonne has over 200 ongoing research projects, covering a broad range of science from studies of the atomic nucleus to global climate change research. With annual funding exceeding \$720 million, the Laboratory has over 3,000 employees, including approximately 1,250 scientists and engineers.

The Director of Argonne National Laboratory shall be executive head of the Laboratory, subject to the direction and supervision of the Board of Governors. The Director will also serve as President of UChicago Argonne, LLC. With support from the University of Chicago, the Director will work with the DOE, and with Laboratory senior management, its Board of Governors and other constituents, to harness the depth of strength at Argonne in staking out a unique future for the Laboratory within the global R&D enterprise. The Director will play a key role in assembling the resources, both public and private, to support the Laboratory. The position will afford the new Director an opportunity to nurture cooperative agreements among industry, members of the national laboratory complex and universities, as well as have a strong voice in shaping national policy on science and technology.

The ideal candidate will have: significant and successful experience managing a large research and development organization; experience working in complex, multi-stakeholder organizations; and excellent communication skills. A Ph.D. in a science or engineering discipline is preferred. Experience in program development with the DOE, DoD, DHS, NASA or other federal agencies with interaction at the Secretary level is preferred.

Please apply electronically at: <http://bit.ly/ArgonneLabDir>

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There's only one

DR. SHIRLEY MALCOM



To Dr. Shirley Malcom, born and raised in the segregated South more than 65 years ago, a career based on her studies in science seemed even less likely than the launch of the Soviet's Sputnik. But with Sputnik's success, the Space Race officially started and, in an instant, brought a laser-like focus to science education and ways to deliver a proper response. Not long after, Dr. Malcom entered the picture.

Although black schools at the time received fewer dollars per student and did not have sufficient resources to maintain their labs at a level equivalent to the white schools, Dr. Malcom found her way to the University of Washington where she succeeded in obtaining a B.S. in spite of the difficulties of being an African American woman in the field of science. From there she went on to earn a Ph.D. in ecology from Penn State and held a faculty position at the University of North Carolina, Wilmington.

Dr. Malcom has served at the AAAS in multiple capacities, and is presently Head of the Directorate for Education and Human Resources Programs. Nominated by President Clinton to the National Science Board, she also held a position on his Committee of Advisors on Science and Technology. She is currently a member of the Caltech Board of Trustees, a Regent of Morgan State University, and co-chair of the Gender Advisory Board of the UN Commission on Science and Technology for Development. She has held numerous other positions of distinction and is the principal author of *The Double Bind: The Price of Being a Minority Woman in Science*.

Of her active career in science, Dr. Malcom says, "I guess I have become a poster child for taking one's science background and using that in many other ways: we ask questions; we try to understand what we find; we consider what evidence we would need to confirm or refute hypotheses. And that happens in whatever setting one finds oneself."

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About NUAA

Nanjing University of Aeronautics and Astronautics (NUAA) is a research-oriented national key university of "211 Project". It also enjoys a well-balanced development of multiple disciplines in engineering, technology, natural sciences, economy, management and social sciences with the characteristics of aeronautics, astronautics and civil aviation. NUAA is qualified to be "Dominant Discipline Innovation Platform of 985 Project" and to independently recruit and receive international students who are granted the Chinese Government Scholarship. Now NUAA consists of 16 colleges with more than 3,000 staff members and approximately 26,000 degree students.

Academia and education at NUAA represent strong capacity among all the universities in China. It has acquired national status through the quality of its excellence research work, especially in the areas of Aerospace Engineering, Mechanics, Electromechanics, Economy and Management, etc.

NUAA gives a warm welcome to excellent experts, scholars and young students from both home and abroad, who are willing to serve the country, dedicate themselves to the development of aerospace science and make contributions to the industrialization, information technology of China. NUAA will provide teachers and researchers with a good academic environment, satisfactory working and living conditions and a stage on which they can put their talents to good use.

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Web: <http://www.nuaa.edu.cn/nuaanew> <http://rsc.nuaa.edu.cn>



Faculty Positions available at Hohai University, Nanjing, China

Hohai University invites applications for faculty positions at the assistant, associate, or full professor level in the area of engineering, science, economics, management, liberal arts, and law. Applicants should have a doctoral degree from a prestigious university. For the complete job announcements and directions on how to apply, visit: rsc.hhu.edu.cn or contact the Department of human resource at 86-25-83786205.

Hohai University, founded in 1915, wins its worldwide reputation on the research of Water Science & Civil Engineering & Environment Engineering. It is a National key university of China, and among the universities of the National "211 Project" and Innovation Bases of the National "985 Project". Hohai University aims to be a research oriented university.



Faculty Positions Available in Southwest University, Chongqing, China

Southwest University is a national key university of the "211" project directly under the Ministry of Education. It is located in Chongqing, the youngest municipality of China. The university hosts approximately 50,000 students, covering undergraduate, postgraduate and other programs. For more detailed information, please visit the website: <http://www.swu.edu.cn/#>

Applications for full-time professors, associate professors and distinguished scientists are welcome. Competitive salaries and start-up funds will be provided to successful candidates, in line with the national Recruitment Program of Young Experts.

The Recruitment Program of Young Experts (i.e. the Plan for Recruiting 1,000 Professorship for Young Talents): The candidates are required to be under the age of 40 and have obtained a PhD degree in a world-renowned university with at least 3 years of research experience abroad, or have obtained a PhD degree in Mainland China with at least 5 years of research and teaching experience abroad. Special offers will be granted to those who have excellent research achievements during their doctoral study.

Further information is available at <http://renshi.swu.edu.cn/rcgzbgbs/>. The Talents Recruitment Office, Southwest University, Beibei, Chongqing 400715, P. R. China. 0086-23-68254265.

Please kindly send applications or nominations in the form of an application letter enclosing a current CV to rencai@swu.edu.cn.



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Assistant Professor (Tenure Track) of Landscape Ecology

The Department of Environmental Systems Science (www.usys.ethz.ch) at ETH Zurich and the Swiss Federal Research Institute WSL (www.wsl.ch) invite applications for an assistant professor position to lead a research group in Landscape Ecology. The professorship will be established as a joint position between ETH Zurich and WSL. The new colleague will be expected to engage in extensive research collaborations with those professorships and research groups at ETH Zurich and WSL that share similar interests, and with other relevant institutions both nationally and internationally. The overall goal of this professorship will be to develop an integrative approach that focuses on the temporal and spatial interactions between processes such as competition, animal and plant dispersal, natural disturbance dynamics and the drivers of environmental change, so as to develop a predictive understanding of how landscape patterns emerge.

The new professor will develop an internationally recognized research program focusing on the interactions between the spatial patterns of land cover and ecological processes, with implications for the provision of multiple ecosystem services. His or her research will contribute to the development of new concepts to assess, predict and manage landscape dynamics at regional scales over decades to centuries. The new professor will be expected to teach undergraduate level courses (German or English) and graduate level courses (English).

This assistant professorship has been established to promote the careers of younger scientists. The initial appointment is for four years with the possibility of renewal for an additional two-year period and promotion to a permanent position.

Please apply online at www.facultyaffairs.ethz.ch

Applications should include a curriculum vitae, a list of publications, a statement of your future research and teaching interests and the names and contact information of three possible referees. The letter of application should be addressed to the **President of ETH Zurich, Prof. Dr. Ralph Eichler**. **The closing date for applications is 15 April 2014**. ETH Zurich is an equal opportunity and family friendly employer and is further responsive to the needs of dual career couples. In order to increase the number of women in leading academic positions, we specifically encourage women to apply.

INDEPENDENT RESEARCH FELLOWSHIPS


John Innes Centre

The John Innes Centre (JIC), Norwich, UK is a world leading centre of excellence in plant and microbial sciences based on the Norwich Research Park. We are inviting applications from outstanding researchers who either hold, or wish to apply for Independent Research Fellowships [such as a BBSRC David Phillips Fellowship (<http://www.bbsrc.ac.uk/funding/fellowships/david-phillips.aspx>), or a Royal Society University Research Fellowship (<http://royalsociety.org/grants/schemes/university-research/>)], to attend a Conference at the JIC on 28th April 2014. At the meeting you will be able to present a talk about your proposed area of research and to discuss your proposals, the development of your group and your future career plans in depth with senior JIC Scientists.

After the Conference we will select and mentor outstanding candidates in writing Fellowship applications and/or offer the opportunity to move existing Fellowships to the JIC. Considerable additional resources will be provided to Fellows by the Centre.

Further details and particulars can be found at <http://www.jic.ac.uk/corporate/opportunities/vacancies/fellows.htm>

Please e-mail a 2-page summary of your research plan, a copy of your CV and arrange for three letters of recommendation to be emailed to dawn.rivett@nbi.ac.uk by Friday 14th March 2014.

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Cell Biology Faculty Positions

The Department of Cell Biology and the Department of Pediatrics at the University of Pittsburgh, School of Medicine seek candidates for Assistant, Associate and/or Full Professor tenure stream and tenured faculty positions. We encourage applications from candidates with a strong record of research accomplishments in the broad area of cell biology, including, but not limited to autophagy, protein and lipid trafficking, intracellular signaling and cytoskeleton. Applications from cell biologists who are using mass-spectrometry as the major approach to address basic questions are also encouraged. Successful candidates will join an interactive, interdisciplinary group of faculty, students and fellows, and enjoy access to the state-of-the-art equipment and facilities at the University of Pittsburgh. Candidates must hold a Ph.D. or an equivalent degree. Highly competitive start-up, compensation and benefits packages are offered.

Curriculum vitae, statement of research interests, two recent most important papers, and e-mail addresses of three references can be sent to: cbprecru@pitt.edu. Review of applications will begin immediately and continue until the position is filled.

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Stony Brook University

Endowed Chair in Theoretical Physics

Stony Brook University seeks a senior faculty appointment in theoretical physics at the level of full professor to occupy the Chen Ning Yang-Deng Wei Chair, now being established. This appointment will be in the C.N. Yang Institute for Theoretical Physics, with affiliation to the Department of Physics and Astronomy. Candidates should have demonstrated exceptional scholarly achievement and potential in research. The ability to attract external funding and leadership potential will also be considered for this position. The position will include an independent research fund and carry a nationally-competitive salary.

The C. N. Yang Institute for Theoretical Physics was established in 1966 and is named for its founding director. It carries on a proud tradition of front-line research and committed education and training. Its faculty, graduates and postdoctoral alumni are active throughout the international scientific community. Current faculty carry on research in a wide range of theoretical physics, including field and string theory, particle phenomenology, statistical mechanics and quantum information. The research interests of candidates for this position may be in any area of theoretical physics, astrophysics and cosmology.

The Institute is an independent unit of Stony Brook University, reporting to the Provost. It has numerous collaborative interactions with the Department of Physics and Astronomy, the Department of Mathematics and the Simons Center for Geometry and Physics, and with other departments at Stony Brook University and with nearby Brookhaven National Laboratory. Stony Brook University is located on the scenic North Shore of Long Island. It has active cultural programs and is convenient to New York City. It has numerous leading graduate programs, and its 1,100 acre campus and 13,500 faculty and staff serve over 24,000 students. The University is a member of the Association of American Universities and co-manager of Brookhaven National Laboratory, a multidisciplinary research laboratory supporting world-class scientific programs utilizing state-of-the-art facilities.

To view application procedure, full position description or to apply online visit www.stonybrook.edu/jobs (Ref. # F-8330-13-11). Nominations and suggestions from our colleagues as well as direct applications are welcome at <http://max2.physics.sunysb.edu/chairsearch/>.

Stony Brook University/SUNY is an equal opportunity, affirmative action employer.



CHEMOINFORMATICS INVESTIGATOR MADISON, WISCONSIN

The Morgridge Institute for Research (MIR) is accepting applications for Investigators whose research focuses on chemoinformatics, the analysis of biomolecular interactions using computation-intensive methods and small molecule probes. We seek researchers who use these approaches to build a fundamental understanding of ligand-target relationships and networks, and to develop laboratory and *in silico* methodologies that significantly widen the scope of chemoinformatics and its impact on other disciplines.

MIR Investigators are full-time, independent research group leaders, expected to develop collegial relationships with other investigators at MIR and the University of Wisconsin-Madison and to participate in selected outreach programs. Formal classroom teaching is not required for a MIR appointment, although training and mentoring of graduate students and postdoctoral researchers is encouraged. MIR provides generous start-up support and commits to substantial ongoing salary support of MIR Investigators.

The Morgridge Institute for Research is a private, nonprofit research institute dedicated to improving human health through interdisciplinary biomedical research, in partnership with the University of Wisconsin-Madison. MIR offers an extraordinary work environment built around a multi-disciplinary fusion of ideas, state-of-the-art facilities, and mentorship by groundbreaking science leaders. www.morgridge.org.

To Apply: Please send a letter of interest, a current CV, a description of your research program and career goals, and include an explanation of why you feel a position that specifically values collaboration and collegiality would be a good fit to compbioinvestigator@morgridge.org. Also, please list three referees who can evaluate your accomplishments and potential, and discuss your creativity, collegiality, and willingness to break new ground.

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Research Positions

Careers Applied Science, Technology, and Engineering Research



INL's Energy and Environment Science and Technology Directorate is seeking outstanding, highly creative and motivated early to mid-level career professionals to join our multi-disciplinary research teams. The Directorate is INL's principal multi-mission organization focused on research to advance clean energy systems, advanced transportation, advanced process technology and related sciences.

Multiple positions are available in the following areas:

- Bioenergy research including biomass characterization, conversion, preprocessing and molecular biology.
- Analytical chemistry specializing in laser spectroscopy and mass spectrometry.
- Materials science and physics with focus on performance in harsh environments, nondestructive evaluation, and radiation based imaging.
- Membrane science with a focus on dense film separation and filtration.
- Geology and Hydrology with a focus on hydraulic fracturing and geothermal evaluation.
- Scientific visualization research in a CAVE environment with a focus on immerse visualization, virtual reality, graphics programming, and large format display technologies.

The Idaho National Laboratory is a science-based, applied engineering national laboratory dedicated to supporting the U.S. Department of Energy's mission in nuclear energy research, science, and national defense. With 3,800 scientists, researchers and support staff, the laboratory works with national and international governments, universities and industry partners to discover new science, develop technologies that underpin the nation's nuclear and renewable energy, national security and environmental missions. The Laboratory is a multi-program national laboratory. It currently performs a range of research and development activities associated with energy and national security. The laboratory currently has more than 150,000 sq. ft. of modern research facilities with a significant amount of wet laboratory space that are supported by a vast array of state-of-the-art research instrumentation. Idaho Falls is conveniently situated near many national treasures such as Yellowstone National Park, Teton National Park, Jackson, WY, etc. For more information about the area, please visit www.visitidahofalls.com and www.visitidaho.org. Interested parties should visit our website at www.inl.gov.

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The Center of Excellence in Environmental Toxicology (CEET) at the University of Pennsylvania Perelman School of Medicine announces the availability of **TWO POSTDOCTORAL POSITIONS** on its Translational Research Training Program in Environmental Health Sciences. The focus of the Center includes research in Lung and Airway Disease, Oxidative Stress/Oxidative Stress Injury; Reproduction, Endocrinology, and Development; and Gene-Environment Interactions.

Postdoctoral applicants must conduct full-time research in translational environmental health sciences. Applicants must be U.S. Citizen or Permanent Resident and would be supported for up to two years. Salary and benefits are commensurate with NRSA approved levels. For further information, application procedure, and list of faculty mentors, please contact e-mail: webster@upenn.edu. The deadline to apply for an appointment beginning July 01, 2014 is April 01, 2014.



FACULTY POSITION in Cancer Biology and Cancer Prevention

The Susan Lehman Cullman Laboratory for Cancer Research in the Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey is seeking an outstanding investigator for a tenure or tenure-track position. Applicants should hold a Ph.D. degree or equivalent and have a strong track record for research in the areas of mechanisms of cancer causation and prevention and cell growth regulation, as evidenced by independent, currently funded research (RO1 or equivalent) and high-impact publications. A strong commitment to teaching undergraduate and graduate students, and postgraduate trainees will be expected. The successful candidate will also be a member of The Cancer Institute of New Jersey. Please send or e-mail curriculum vitae, brief research plan, current and past grant support, and the names and addresses of three references to: **Dr. Suzie Chen, Chair of the Search Committee**, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, 164 Frelinghuysen Road, Piscataway, NJ 08854-8020, e-mail: bachorik@pharmacy.rutgers.edu.

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